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РЕЗЮМЕ

СВОБОДНЫЕ И N-ОКСИДНЫЕ АЛКАЛОИДЫ SENECIO VULGARIS L. ИЗМЕНЕНИЕ СОДЕРЖАНИЯ АЛКАЛОИДОВ В РАЗНЫХ ОРГАНАХ В ПЕРИОД ВЕГЕТАЦИИ

А. КЕРИ

Содержание общего, свободного и N-оксидного алкалоида растений **Senecio vulgaris L.** определялось в разных органах в период развития. Найдено, что **Senecio vulgaris L.** в период всего развития характеризуется предоминированием свободных алкалоидов, однако N-оксидные алкалоиды могут быть обнаружены во всех органах в течение всего вегетационного периода.

НАСЛЕДСТВЕННО-ПАТОЛОГИЧЕСКИЕ И ПЛЕМЕННО-ГИГИЕНИЧЕСКИЕ ПРОБЛЕМЫ ОБРАЗОВАНИЯ БЛИЗНЕЦОВ У КРУПНОГО РОГАТОГО СКОТА

Д. ХАМОРИ

Среди некоторых попыток повысить продукцию мяса образование близнецов стало интенсивно изучаемой областью. Соответственно изученным литературным данным и официальным данным венгерского племенного реестра были анализированы биологические и генетические трудности. В 1966 году из 120 375 отелившихся венгерских пёстрых коров 4360 имели близнецов (3,62%), в результате чего было получено 8727 телят, затрудненные роды составили 31,65%, от этих родов 998 телят оказались мертвыми при рождении (11,43%), 2121 погибли до отнятия (27,44%) и вследствие последующей стерильности 731 коров нужно было выбраковать (16,76%). В 1969 году из 160 864 отелившихся коров 5435 имели близнецов (3,37%), в результате чего было получено 10 882 телят, затрудненные роды были в 37,38%, от этих родов 1562 телят оказались мертвыми при рождении (14,36%), 2829 погибло до отнятия (30,35%) и 987 коров было выбраковано вследствие последующей стерильности (18,16%). Склонность к возникновению близнецов сложный (полимерный) признак, который демонстрировался в семейно-потомственном исследовании, но наследуемость его является низкой (h^2 0,04—0,25), хотя этот показатель меняется среди различных пород. Гетеросексуальные близнецы у венгерских пёстрых коров, зарегистрированные как женские, показывают 96% стерильность (интерсексуальность), 21,8% из них не могли быть использованы для получения потомства (на племя). Хромосомные ненормальности были тоже обнаружены среди этих близнецов (химеризм), у женских близнецов идентичного пола 45,4% не оплодотворялись в результате врожденной стерильности. Автор указывает на то, что с точки зрения здоровья животных и экономики ущерб, связанный с возникновением близнецов, является нежелательным. Если бы биологические трудности могли быть преодолены, образование близнецов осталось бы техникой с некоторыми невыгодностями, поэтому тщательный экономический анализ необходим до введения в общую практику.

РОЛЬ ЛИШАЙНИКА В ОБОРОТЕ АЗОТА СТЕПНЫХ ДЕРНОВЫХ СООБЩЕСТВ

К. ВЕРШЕГИ, И. Э. ЛАНГ

На песчаной дернине в течение двух лет (май 1970—апрель 1972) изучали сезонное изменение содержания общего азота живущих в почве ксерофитов лишайников **Cladonia magyarica**, **Cl. furcata**, **Cl. convoluta** и **Parmelia pokornyi** и роль лишайников в обороте азота сообщества. В колонии лишайника общее количество азота обычно ниже одного процента и показывает сильное сезонное изменение. Различие, обнаруженное у разных видов, зависит от структуры колонии лишайника. Пропорция и роль разных видов в обмене веществ азота сообщества определяются соотношением массы. Их роль в **Brometum tectarum** больше, чем в **Festucetum vaginatae**. Интенсивность оборота азота меняется в зависимости от вида и года.

ЭФФЕКТ 6-МЕТИЛУРАЦИЛА И 2-ХЛОРЭТИЛФОСФОРНОЙ КИСЛОТЫ НА ФРУКТИФИКАЦИЮ И УРОЖАЙ РАЗЛИЧНЫХ ЛИНИЙ ШАМПИНЬОНА AGARICUS BISPORUS (LANGE/SINGER)

И. РИМОЦИ, И. ВЕТТЕР

Авторы в своих опытах изучали эффекты 6-метилурацила и 2-хлороэтилфосфорной кислоты на фруктификацию и урожай различных сортов шампиньона **Agaricus bisporus (Lange/ Singer)** в условиях мелкого производства. Изученные сорта (выведенные линии) были взяты частично из самых распространенных в производстве сортов (в предыдуших годах: Пц-17, в настоящее время: Д-13, Ф-1), частично из сортов, которые в настоящее время ещё мало используются (ШО-9, З-3). Установлено, что оба действующие вещества — добавленные к компосту — в значительной мере ускоряли появление плодового тела по сравнению с необработанными контрольными сортами. Под влиянием обработок фруктификационные периоды стали более выравненными, особенно в случае сортов ШО-9 и З-3. Действующие вещества в значительной мере повысили средний урожай линий Пц-17 и в меньшей мере Д-13, Ф-1, ШО-9, З-3. Повышение среднего урожая использованных сортов показало максимальную величину при разных концентрациях в среднем 15—30%, в некоторых случаях достигая 35—50% стимуляции. Примененные 2-хлороэтилфосфорная кислота и метилурацил, имеющий характер цитокинина, влияли на фруктификацию сортов, обладающих различными морфологическими и физиологическими свойствами. Этот факт, по-видимому, доказывает регулирующее действие роста и развития применённых веществ. Результаты опытов — после дальнейших исследований — по-видимому могут быть использованы в условиях крупнохозяйственного грибоводства. Их физиологические аспекты могут способствовать изучению фруктификации и действия 6-метилурацила и-2-хлороэтилфосфорной кислоты.

ОБРАЗОВАНИЕ РАФИД-ИДИОПЛАСТОВ В ВОЗДУШНОМ КОРНЕ MONSTERA DELICIOSA LIEBM.

А. КОВАЧ, ИН. РАКОВАН

Авторы изучали образование и дифференциацию рафид-идиопластов в воздушном корне **Monstera deliciosa**. Они установили, что эти клетки образуются после инеквиального деления, имеют крупные клеточные ядра и активную цитоплазму. Полости с кристаллами располагаются в несколько рядов линейно и идиопласты, расположенные на определенном растоянии одни над другими, могут соприкасаться вследствие более быстрого роста, чем их окружающие.

ИССЛЕДОВАНИЯ ПО ОПРЕДЕЛЕНИЮ ПОТРЕБНОСТИ ЛИЗИНА И МЕТИОНИНА I

ОПРЕДЕЛЕНИЕ ОБЕСПЕЧЕННОСТИ АМИНОКИСЛОТАМИ ОТКОРМОЧНЫХ СВИНЕЙ В ПРОИЗВОДСТВЕННЫХ ОПЫТАХ С ПОМОЩЬЮ ИЗУЧЕНИЯ АЗОТООБОРОТА

М. СЕЛЕНИ-ГАЛАНТАИ, Д. ЕЧАИ, Б. ЮХАС

Опыты по групповому откармливанию поросят проведены с венгерской крупной породой мясного типа и сделан также анализ азотооборота при использовании кормовой смеси в групповом опыте с белыми крысами и свиньями. Процентное отношение лизина и метионина в кормовой смеси менялось. Найдена тесная корреляция ($r = 0,9716$) между потребленным количеством лизина и показателями азоторавновесия. Установлено, что анализ азотооборота пригоден для определения потребности аминокислот у свиней. Определено также, что к корму, содержащему 10% сырого белка и 72 кг/ц крахмального эквивалента, нужно дать 0,9% лизина и 0,2% метонина. Результаты групповых опытов откармливания и изучения азотооборота с достоверностью подтвердили эти показатели потребности аминокислот.

СТИМУЛИРУЮЩИЙ ЭФФЕКТ ОПРЫСКИВАНИЯ УДОБРЕНИЕМ, СОДЕРЖАЩИМ МАГНЕЗИЙ, НА СИНТЕЗ БЕЛКА

А. Ш. КИШШ, Б. И. ПОЖАР

Опрыскивание листьев удобрением, к которому был прибавлен ион магнезия, в значительной мере стимулировало использование аминоазота белком. Магнезий особенно значительно повышал накопление азота в белке в первые три часа после обработки. Стимулирующий непосредственный эффект магнезия в синтезе белка, повидимому, может быть сведен к двум факторам. Влияние одного из них заключается в поддержании полирибосомальных структур, что является лимитирующим условием синтеза пептида, с другой стороны, в биоэнергетическом отношении магнезий в реакциях аминирования как активатор снижает требование изменения свободной энергии в превращении. Эти два механизма действия дают удовлетворяющее объяснение для теоретической интерпретации стимулирующего действия иона магнезия на синтез белка.

НЕКОТОРЫЕ НАБЛЮДЕНИЯ ПО ФРАКЦИИ СПЕРМОЗИНА

СПЕКТР ВОЗБУЖДЕНИЯ И ФЛУОРЕСЦЕНЦИИ И АМИНОКИСЛОТНЫЙ СОСТАВ ФРАКЦИЙ СПЕРМОЗИНА И АКТОСПЕРМОЗИНА

Ш. ФАЗЕКАШ, И. ВЕРЕШ, И. КАША, А. ПАТТИ, Е. ТИХАК

В наших опытах исследовались определенные фракции актоспермозина и хроматографические фракции спермозина. В ходе фракционирования следили за диспропорционированием хвостовой части сперматозоида и представляем это на снимках, полученных с помощью светового и электронного микроскопов. Были изучены спектры возбуждения и флуоресценции хроматографических фракций актоспермозина и спермозина, а также состав аминокислоты. Наши результаты показывают, что спектр возбуждения актоспермозина ограничивается довольно узкой полосой, но спектр флуоресценции даже вблизи вершины имеет широкую поверхность, последнее демонстрирует, что его компоненты не однородные. До хроматографирования спектр фракции возбуждения и флуоресценции спермозина проявляет гетерогенность и это подтверждает шесть фракций отделения. Фракции III, IV и V имеют значительную активность ATP, активность фракции II ещё не определена. Вопреки отделению спектр возбуждения и флуоресценции некоторых фракций ещё указывает на гетерогенность. В составе фракции IV и V спермозина и актоспермозина встречается метилированный лизин, а именно актоспермозин имеет примерно 1 М%, фракция IV 0,75 М% и фракция V 1,22 М% N-метилированный лизин. Сравнительные стандартные хроматограммы основной аминокислоты показывают, что метилированная аминокислота фракций актоспермозина представляет собой ε-N-триметил лизин.

РЕАКЦИЯ МАЛОИЗВЕСТНОГО РАСТЕНИЯ LABIATAE К ДВЕНАДЦАТИ ВИРУСАМ

Й. ХОРВАТ

В опытах искусственного заражения по изучению чувствительности **Ocimum canum Sims** (семейство: **Labiatae**) к вирусам было установлено, что это растение локально чувствительно к вирусу **tobacco rattle** (R/1 : 2,3/5 : E/E : S/Ne), далее локально и систематически чувствительно к вирусу **cucumber mosaic** (R/1 : 1/18 : S/S : S/AP), к вирусу **potato aucuba mosaic** (∗/ ∗: ∗/ ∗:E/E : S/Ap), к вирусу-X **potato** (R/1 : ∗/6 : E/E : S/Fu), к вирусу **tobacco mosaic** (R/1 : 2/5 : E/E : S/ ∗) и к вирусу **tobacco ring spot** (R/1 : 1,8/42 : S/S : S/Ne). В опытах заражения **Ocimum canum Sims** оказался иммунным к шести вирусам/вирус **bean common mosaic**, ∗/ ∗: ∗/ ∗: E/E : S/Ap; вирус **M potato**, ∗/ ∗: ∗/ ∗: E/E : S/Ap; вирус **S potato**, ∗/ ∗: ∗/ ∗: E/E : S/Ap; вирус **У potato**, ∗/ ∗: ∗/ ∗: E/E : S/Ap; и вирус **radish mosaic**, R/ ∗/: ∗/ ∗: S/S : S/Cl; **turnip yellow mosaic**, (R/1 : 1,9/37 : S/S : S/Cl). **Ocimum canum Sims** как **screening host plant** может быть использован для сепарации некоторых вирусов, и особенно пригоден для идентификации вируса **cucumber mosaic**.

ВОЗДУШНАЯ СРЕДА И ТОЛЕРАНТНОСТЬ POLYGONATUM ODORATUM (MILL.) DRUCE В ЕСТЕСТВЕННЫХ СООБЩЕСТВАХ

Г. ФЕКЕТЕ

Автор изучал реакции нетто фотосинтеза **Polygonatum odoratum** на одном месте Будайских гор (средневенгерские горы) в естественных сообществах. В изученной системе многолетние порослевые колонии достигли самого большого распространения и числа в типе **Orno-Quercetum Brachypodium** в более всего богатой светом луговой степи, и мало особей **Orno-Quercetum** встречалось на месте произрастания **Vicia sparsiflora** с бедным светом. Световой климат места, где произрастает лес, автор характеризует распределением частоты показателя интенсивности освещения и в этой связи изучает кривые света **Polygonatum**. В отношении дневных ходов у растений луговой степи брутто фотосинтеза до 32—33 °C компенсирует дефицит дыхания и для него характерна по сравнению с другими типами возобновляющаяся фотосинтетическая активность, которая может быть значительна. Линеарная часть световой кривой лесной особи, выросшей на месте произрастания с бедным светом, является менее крутой, чем на месте с богатым светом (**Brachypodium**), суммарное содержание хлорофилла на единицу поверхности также ниже и показатель освещения места произрастания в десять раз слабее используется, чем чаще всего встречаемый показатель освещения своего места произрастания у особи, растущей на месте произрастания **Brachypodium**. Плохая теневая адаптация вида может быть экологическим лимитирующим фактором.

ВЛИЯНИЕ ВРЕМЕНИ ПОСАДКИ НА ПРОДУКЦИЮ СУХОГО ВЕЩЕСТВА ТОМАТА И ИСПОЛЬЗОВАНИЕ СВЕТОВОЙ ЭНЕРГИИ

Ш. БАРООВА, И. ХОРВАТ

Время посадки влияет на сухой вес и ход накопления сухого вещества. В случае ранней посадки (май) сухой вес обоих изученных сортов помидора — кечкеметский консервный и кечкеметский карлик — примерно в течение 3—4 месяцев увеличивается, потом в незначительной мере снижается (часть листьев опадала). В случае поздней посадки (июнь-июль) повышение сухого веса продолжается в течение 4—5 месяцев, но чем позднее сделана посадка, тем ниже сухой вес растений. Самый большой урожай у обоих сортов был получен в случае ранней посадки. Сорт кечкеметский консервный в бóльшей степени реагировал на время посадки, чем сорт кечкеметский карлик. Концентрация всех углеводов в сорте кечкеметский консервный обычно выше, чем в сорте кечкеметский карлик, но концентрация общего азота в обоих сортах приблизительно одинаковая. Использование энергии двумя сортами разное. Эта разница может быть связана с морфологическими признаками и структурой популяции двух сортов (высота, сомкнутость популяции, площадь листьев). Использование энергии скорее определяется сомкнутостью популяции, чем площадью листьев. Индекс оптимальной площади листьев равен примерно 0,3 у сорта кечкеметский карлик, и 0,6 у сорта кечкеметский консервный.

СРАВНИТЕЛЬНОЕ АНАТОМИЧЕСКОЕ ИЗУЧЕНИЕ НА LOTUS CORNICULATUS AGG. III.

О. С. БОРОШ

При изучении анатомии корня, проводимом на дикорастующих и культивируемых таксонах **Lotus corniculatus agg.**, можно установить, что первичный корень, характеризующий таксоны, имеет тип **triarch** и **tetrarch**. Вторичное утолщение начинается через 6—8 недель. Обнаружено различие на уровне мелкого вида и подвида в тканевой структуре корней, образующихся в ходе вторичного утолщения. Таким образом, определяющим характером для различных таксонов **Lotus** является вторичная тканевая дифференцировка.

ВОЗМОЖНЫЕ ПУТИ МОРФОГЕНЕЗА В КУЛЬТУРЕ КАЛЛЮСНОЙ ТКАНИ ВЫСШИХ РАСТЕНИЙ

Л. ХЕСКИ

Цель нашей работы изучить, какие процессы дифференцирования клетки и ткани протекают в каллюсной ткани до появления растений (побегов, корней). По нашим наблюдениям образованию одних и тех же органов могут предшествовать совершенно различные процессы дифференцирования. Те же концентрации гормонов в каллюсных тканях разных видов растений, а также разные концентрации гормона и взаимосвязи в каллюсной ткани одного и того же вида могут индуцировать адвентивный эмбриогенез или дифференцирование конуса нарастания. В статье описывается адвентивный эмбриогенез, образование конуса нарастания корня и стебля, а также органогенез. На основании своих и последних результатов других исследователей наметили те возможные варианты дифференцирования клетки и ткани, органогенеза, которые делают возможным создать растения из недифференцированных каллюсных клеток.

Our sincere regards to Professor Pál Greguss
on the occasion of his 85th birthday

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FREE AND N-OXIDE ALKALOIDS OF SENECIO VULGARIS L. CHANGES IN THE CONTENTS OF ALKALOIDS IN VARIOUS ORGANS DURING THE VEGETATION PERIOD

By

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The total and particularly the free and N-oxide alkaloid contents of *Senecio vulgaris* L. plants were studied in the various organs during their development. It was found that *Senecio vulgaris* L. is characterized during the whole development by the predominance of free alkaloids, however, N-oxide alkaloids can be found in all the organs throughout the whole vegetation periods.

Introduction

In the last decades several teams of research workers have set themselves the aim of studying the plant species containing *Senecio* alkaloids, from phytochemical aspects. The pyrrolysidine alkaloids, owing to their peculiar ring system, represent quite an interesting group of alkaloids, and have attracted the attention of organic chemists and analysts. Adams–Govindachari (1949a, b), Shun *et al.* (1960), Kohlmünzer *et al.* (1971). This group of compounds possesses a definite biological activity. The hepatotoxic property of these alkaloids has been known for a long time; their anti-tumour effect that became known mainly from the treatises of Culvenor (1962, 1968), Kupchan–Doskotch (1964), Kupchan–Suffness (1967) and the spasmolytic action of the only slightly toxic platiphylline utilized in therapy by research workers: Muravyeva (1966) have been observed only recently.

At the same time only a few literary data are available concerning the problems of the location of the pyrrolysidine alkaloids in the plants, of the dynamics of their accumulation, and of their distribution during the vegetation period (Muravyeva, 1966).

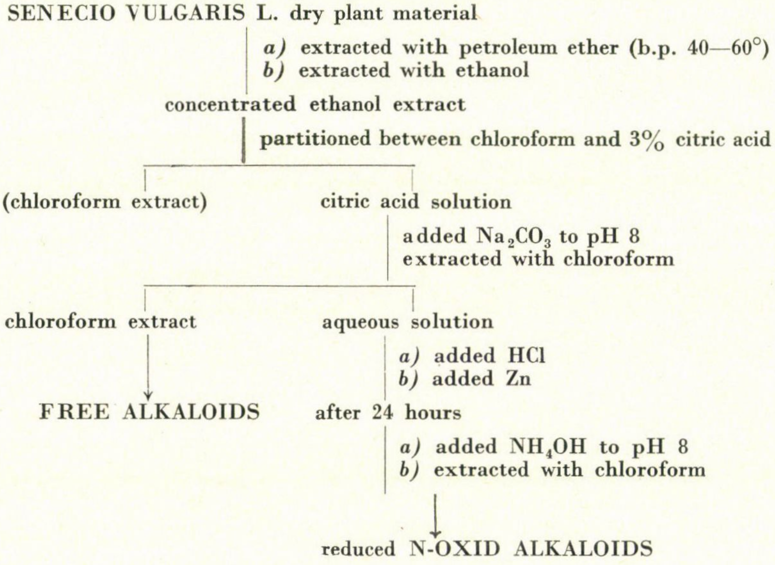
In the course of the complete phytochemical screening test of *Senecio vulgaris* L. that is a common weed in Hungary, a few correlations were observed between the quantitative distribution of the compounds of various type. My observations raised the interest in a detailed study of the occurrence of the alkaloids.

Material and method

For the present investigations plants originating from pure-species seed material of *Senecio vulgaris* L. grown in field plots were used. Phytochemical control tests were performed with a part of the experimental material. The significant part of this material was collected in seven instances during the vegetation period, and the samples were used for alkaloid tests in the individual organs. In the course of the isolation procedures corresponding to the different conditions of solubility of the free and N-oxide alkaloids were applied according to the scheme presented in Table 1.

Table 1

Extraction procedure of free and N-oxide alkaloids from Senecio vulgaris L.

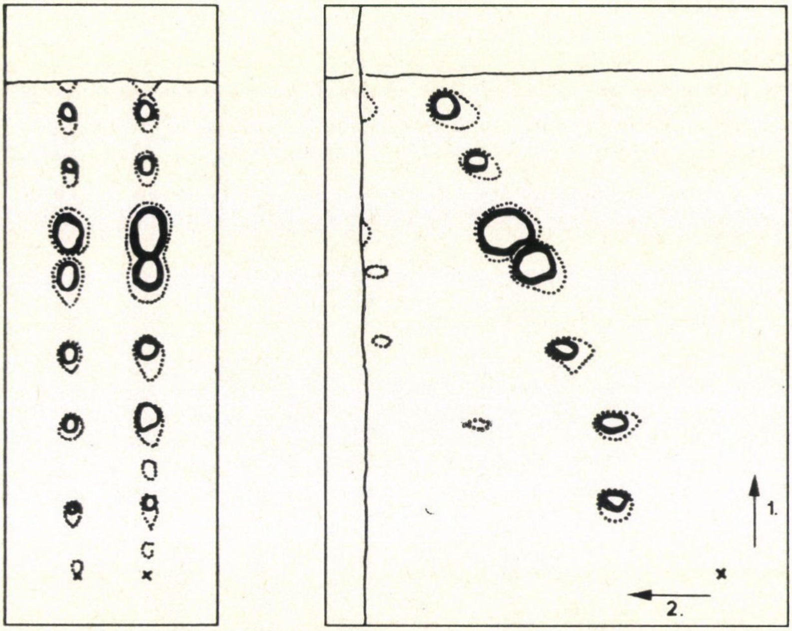


The free alkaloids were prepared from the chloroform extract whereas the alkaloids corresponding to the N-oxide alkaloids from the aqueous phase, subsequent to reduction with zinc (KUPCHAN—DOSKOTCH 1964).

At the orientative thin-layer chromatographic investigation of the crude alkaloid mixtures two main spots concerning Rf values adjacent very closely to each other, further one more significant and two to six weak alkaloid spots were observed. Figure 1 shows the schemes of thin-layer chromatograms on Kieselgel-G plates treated with Dragendorff reagent according to Munier and Machebaeuf, and with Dragendorff reagent sensitized by processing with 0.1 n sulphuric acid, obtained by one-dimensional and two-dimensional development (STAHL 1969).

The alkaloids corresponding to the main spots were purified by repeated column chromatography and preparative layer chromatography, and then isolated. By means of conventional analytical methods, spectroscopical processes, ultraviolet and infrared spectral investigations, analysis of hydrolysis products and the ultraviolet spectra of the corresponding necinic acids it could be stated that in accordance with the literary data, the main alkaloids of the examined plant material of the cultivated *Senecio vulgaris* L. are senecionine and seneciphylline and their N-oxides (BULL *et al.* 1968).

Owing to the minute amounts of materials available it was not possible to study the further components more thoroughly. From the plant material of the cultivated *Senecio vulgaris* L. that had been controlled phytochemically in the way briefly described above, samples were taken at seven dates in the same hour of the day during the vegetation period. At the dates of sampling the stages of development of the plants were as follows; cotyledonous state



n-butanol-acetic acid-water. 1. n-butanol-acetic acid-water; 2. methanol.

Reagent: modified Dragendorff (+0,1n H_2SO_4)

Fig. 1. Schemes of thin-layer chromatograms on Kieselgel-G plates, treated with Dragendorff reagent

(1), development of leaf rosetta (2), tillering (3), budding (4), complete flowering (5), fruit production (6), wilting (7).

The plants were separated into organs; root, stem, leaves, and generative parts, and complete plants were also collected for the determinations of total alkaloid content. The fresh weight and then the dry weight were established as averages calculated from 100 plants. Alkaloid extracts were prepared from the dried powdered substances in the way described above.

The quantity of total free alkaloids and of total N-oxide alkaloids was measured by titration in a non-aqueous medium by the method of the VI. Hungarian Pharmacopoeia. The combined amount of alkaloids of different oxidation states was established by calculation and expressed as seneciphylline corresponding to the overall formula $C_{18}H_{23}NO_5$.

An ERI-65 ZEISS Densitometer was used for the densitometric investigations.

The plates prepared by the above specified method but in order to attain better separation of the mentioned two main components by allowing them to run repeatedly were evaluated directly by the densitometric method. By means of the integral curves of the densitograms geometrical evaluation was carried out, and the ratio of the individual spots was referred to the quantity of all the Dragendorff positive spots.

Results

The alkaloids of *Senecio vulgaris* L. had been studied by several authors, thus quite interesting literary data were available (Shun *et al.* 1960, Gharbo—Habib 1969). Shun *et al.* (1960) were the first, later followed by others who reported that the simultaneous occurrence of free and N-oxide alkaloids is characteristic of this plant species as well. However, quantitative data are rather scarce in the literature, and they refer only to whole plants.

On the basis of my analyses the changes in the contents of free N-oxide and total alkaloids during the development of the plants are shown in Figure 2.

It can be seen that both the free and N-oxide alkaloids are present in the plant at all the tested dates of the vegetation period. The total alkaloid content is the lowest in the cotyledonous state, followed by an intensive rise at the time of development of the leaf rosetta and of the tillering stage. Subsequently the level decreases at budding time, remains at a constant level with slight deviations even exhibiting a small maximum value, to decrease again significantly in the stage of wilting.

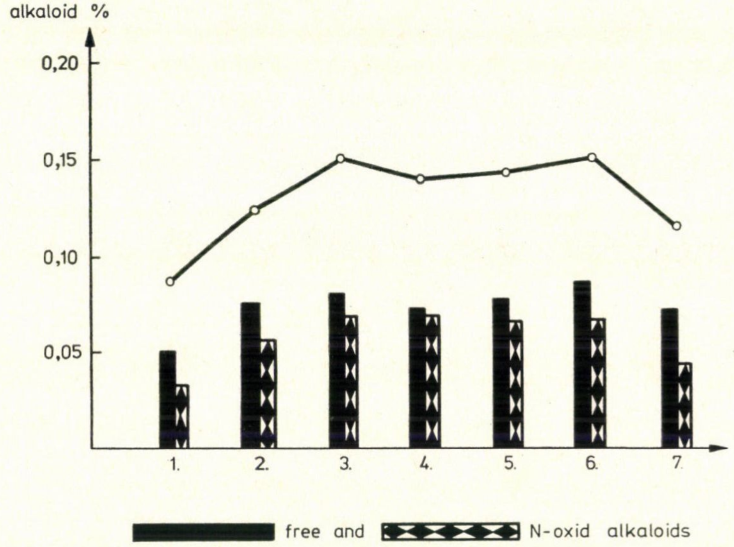


Fig. 2. Changes of total, free and N-oxide alkaloid contents of *Senecio vulgaris* L. during ontogeny. (1—7 developmental phases: —— = total alkaloids.)

The separately measured amounts of free and N-oxide alkaloids show more mobile dynamics. The examined plant material of *Senecio vulgaris* L. is characterized by the predominance of free alkaloids at all stages of the vegetation period though in the medium stage of the vegetation period they only exceed the amount of N-oxides slightly. The relatively lowest contents of N-oxide alkaloids were measured at the beginning of the vegetation period and even in the stage of wilting. The diagram also shows that the content of total alkaloids of the analysed samples referred to the dry matter content ranged from 0.08 to 0.16% in the examined period. On the basis of my investigations of the plant samples according to similar principles and methods,

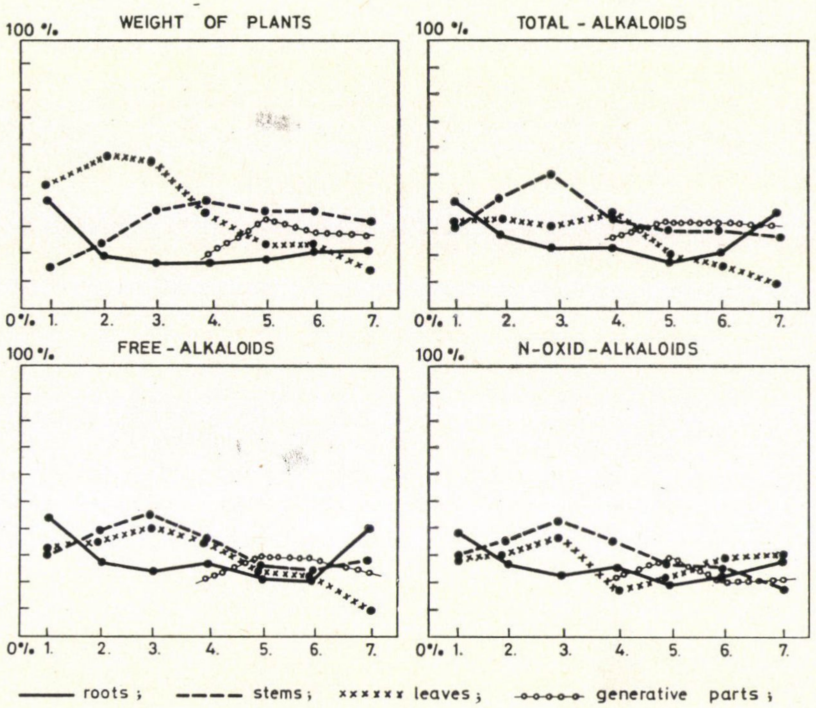


Fig. 3. **Percentage distribution of the total, free and N-oxide alkaloid contents and of the total weight of plants during ontogeny. (1—7 developmental phases.)**

carried out with the plant organs separated from the samples it was possible to extend my preceding observations.

Figure 3 shows the percentage distribution of the total, free and N-oxide alkaloids and of the total weight of the plant between the individual plant organs during the vegetation period.

It can be seen in this series of diagrams that the distribution of alkaloid contents between the various organs deviates, according to expectations, from that of the total weight of the plants. It is striking that in the first two thirds of the vegetation period the herb, particularly the young stem and the leaves, contain the major part of the alkaloid content of the plant. At the very beginning and at the end of the vegetation period the alkaloid content of the roots in relation to the total content is higher, and the alkaloid content of the leaves definitely decreases. In the accumulation of alkaloids in the generative parts the smallest alteration was experienced during the vegetation period. The dynamics of the accumulation of free and N-oxide alkaloids in the individual organs exhibited no essential differences from each other. The decrease of the ratio of N-oxides was more pregnant in the last section of the vegetation period than that of free alkaloids, particularly in the root drogues. In the medium section of the vegetation period the participation ratio of N-oxide alkaloids in the leaves and stem parts was higher. In my further investigations the ratios of seneciphylline senecionine and further components showing positive reaction with the Dragendorff reagent were studied. Figure 4 shows the scheme of a densitogram whereas Table 2 the percentage data of the evaluation of free and N-oxide alkaloids in the case of leaf samples.

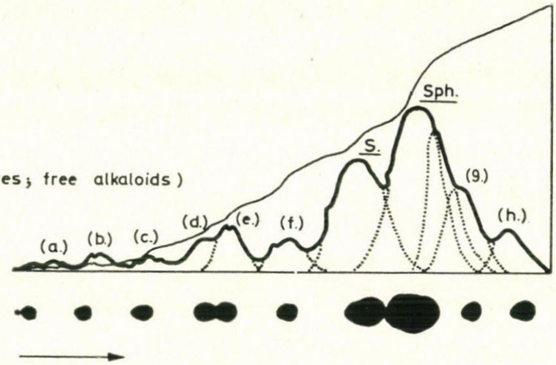


Fig. 4. Scheme of a densitogram of free alkaloids in leaves of *Senecio vulgaris* L.

It must be noted that no essential deviations were experienced either in the ratio of seneciphylline and senecionine in the individual organs or during the vegetation period.

It appears from the data that the ratio of seneciphylline significantly exceeded that of senecionine throughout. More significant differences were observed, however, in the occurrence and ratio of the minor components.

Conclusion

Senecio vulgaris L. plants cultivated under field conditions and controlled from a phytochemical aspect were studied during their development. It was found that *Senecio vulgaris* L. is characterized during the whole development by the predominance of free alkaloids. However, N-oxide alkaloids can be found in all the organs throughout the whole vegetation periods. On the basis of the densitometric measurements the ratio of senecionine and seneciphylline does not alter either in the various organs or during the development of the plants.

Table 2

Percentage data of the evaluation of free and N-oxide alkaloids in case of leaf samples. (1—7 developmental phases Sph = seneciphylline S = senecionine a—h = other Dragendorff-positive spots.)

		Sph.	S.	(a.)	(b.)	(c.)	(d.)	(e.)	(f.)	(g.)	(h.)
Free		%									
alkaloids	1.	60	20	+	+	++	++	+	+	—	++
	2.	75	14	—	+	+	+	+	±	—	+
	3.	74	16	—	—	+	+	+	+	—	+
	4.	70	20	—	—	+	+	+	+	—	+
	5.	72	20	—	—	—	+	+	+	—	+
	6.	65	22	+	±	+	++	+	+	—	—
	7.	63	20	+	±	+	+	++	+	+	+

		Sph.	S.	(a.)	(b.)	(c.)	(d.)	(e.)	(f.)	(g.)	h.)
N-oxid		%									
alkaloids	1.	65	15	+	+	++	+	+	+	+	++
	2.	74	14	—	—	+	+	+	+	—	+
	3.	72	15	—	±	+	++	+	+	—	+
	4.	70	18	—	+	+	+	+	+	—	+
	5.	70	19	—	±	+	+	+	+	—	+
	6.	65	20	+	±	+	++	+	+	—	+
	7.	60	20	+	+	+	++	++	+	+	+

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THE HEREDITARY-PATHOLOGICAL AND BREEDING HYGIENIC PROBLEMS OF TWINNING IN CATTLE

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Among the several efforts to increase beef production twinning has become an intensively studied field. According to data from the literature, studies and data of the official Hungarian breeding-register the biological and genetical difficulties have been analysed. In 1966 4360 twinnings (3.62%) of 120,375 Hungarian Spotted cows resulted in 8727 calves; laborious parturition was observed in 31.65% of the cases: from those delivered 998 were dead at birth (11.43%); 2121 died up to weaning (27.44%) and 731 cows had to be eliminated for subsequent infertility (16.76%). In 1969 5435 twinnings (3.37%) of 160,864 cows resulted in 10.882 calves; laborious parturition was observed in 37.38%; from those delivered 1562 were dead at birth (14.36%); 2829 died up to weaning (30.35%) and 987 cows were eliminated for subsequent infertility (18.16%). Disposition to twinning is a complicated (polymeric) trait which has been demonstrated in family-progeny studies, but its inheritance is low ($h^2 = 0.04—0.25$) although it differs among the various breeds. Heterosexual twins in the Hungarian Spotted cattle registered as female showed infertility in 96% (intersexuality), 21.8% of these could not be used to mast. Chromosome abnormalities were also observed among these twins (chimerism); in female twins of identical sex 45.4% could not be fecundated due to congenital infertility. The author indicates the health and economic disadvantages tied up with twinning in cattle. In the dairy breeds bull-offsprings from twinnings are not advised to be introduced to breeding. If the biological difficulties could be overcome twinning would still remain a technique of several disadvantages and a careful economic analysis is indispensable before introduction to the general practice.

Introduction

In the past few years in order to increase beef production research has orientated towards increasing the number of twin births. In the uniparous domestic animal species this effort encountered numerous biological difficulties.

Recently twinning has gained certain importance in cattle. While in countries with intensive dairy husbandry the actuality of milk production has decreased, the interest of research workers in animal breeding has turned to beef production by increasing the number of twinnings.

The frequency of twinning in the uniparous bovine varies according to breeds and herds. Results published in the literature refer to 0.06—29.4% the latter figure was obtained in a Simmenthal breeding herd (Hansen 1968).

It has been known long since that certain cows give birth to twins repeatedly in their lifetime. In a study of 38,000 deliveries of 15,389 cows it was concluded that frequency of twinning increased with the number of calvings, among cows delivering for the first time it was 0.54% and for the 5th calving it increased to 3.37%.

Having taken into consideration these facts the heritability is approximately 0.25. The mode of inheritance of twinning was described by several authors as a simple recessive trait with incomplete penetration.

To our present knowledge the disposition to twinning is a complicated (polymeric) trait: the development and maturation of numerous follicles in the ovaries as well as polyovulation are influenced by environmental factors too.

The greatest part of multiple conceptions occurs as simultaneous fecundation, polyembriony being relatively rare. Monozygotic twins are approximately 5 per cent of twinnings; and a high 24.59% frequency was published only from Japan in black-spotted cattle. Multiple ovulations were provoked experimentally in several animal species by injecting gonadotropins so it is reasonable to assume that in spontaneous polyovulations the gonadotropin levels are increased which results in multiple conceptions. In cattle the fertilized ova, after polyovulation, may survive as long as 30 days, although the greater the number of fertilized ova the higher the embryonal mortality after that time. Cystic ovaries are most frequently found in dairy cattle breeds and the appearance of nymphomany is a consequence of an altered endocrine system. In beef cattle breeds the incidence of nymphomany is rare, which supports their better-balanced endocrine system.

Material and methods

In our biometric research 96% of the twinnings was dizygotic, that means, their genetic background is quite different. According to the studies even in the cows genetically disposed to twinning, polyovulation does not always result in twinning. The simultaneous fecundation may be influenced by the quality of the semen. The implantation of the fertilized ova is also influenced by the physiological environment prevailing in the uterine epithelium, the embryo is often resorbed or one of the twins dies in the uterus and is aborted. The viability of the twins is greatly influenced by management and feeding factors, the health condition of the dam, different infection etc.

However, experience argues in favour of genetical disposition to twinning;

1. There is a difference in the incidence of twinning among the different breeds. In certain herds the number of twinnings varies even within the same breeds (LUSH 1925). Lush analyzed data for 1.5 million calvings and demonstrated that the incidence of twinning is related to breeds; in the Black-Spotted 4.6%, Norwegian Hornless 2.9%, Danish Red 2.87%, Jersey 1.18%.
2. The dams born as twins give birth to twins more frequently than those from single calving within the same herd (JOHANSSON 1932, RABE 1961) but the reproducibility of twinning is little (LABHSETWAR *et al.* 1962).
3. In cow families with a disposition to twinning the repeated occurrence of the phenomenon can be followed up to 4 or 5 generations (HÁMORI 1970).
4. It is generally agreed that careful selection to twinning may be successful (BRODAUF 1963, HOLTZ *et al.* 1970): but on the other hand, in Virginia, selection for twinning was performed for some 25 years without any noticeable results.
5. The genetical disposition to twinning transmitted by the sire is demonstrated by the fact that the daughters of certain bulls give birth to twins in a much higher proportion (LUSH 1925, etc.).

In 2862 cow descendants (offsprings) of 63 British-Friesian bulls used in artificial insemination 2.8% twinning occurred but 8.8% difference could be demonstrated between certain bull-families.

The great number of publications dealing with the genetics and incidence of twinnings refers to the fact that the mode of inheritance is not a simple one in the uniparous species, and several papers clearly indicate that the familiar disposition to twinning is rather frequent in cattle.

The lowest incidence of twinning could be demonstrated in beef cattle herds; it takes 0.5% in the Hereford and Aberdeen-Angus cows. This supports the low inheritance of disposition to twinning in the beef cattle breeds. In the United States injection of pregnant mare's serum on two occasions increased the frequency of twinnings in beef cattle, but no response was observed with the gonadotrophin treatment of beef cattle in England (Rowson *et al.* 1969).

Results

In dual purpose and dairy breeds the number of twinnings is rather high and among these the Simmenthal breeds and crosses have the highest incidence as it is shown in Table 1.

Table 1

The frequency of twinning in different breeds

Breed	Number of calvings studied	Twinnings	
		number	%
Simmenthal	12,625	582	4.61
Hungarian Spotted 1966	120,375	4360	3.62
Hungarian Spotted 1969	160,864	5435	3.37
Swedish Friesian	24,670	820	3.32
Swedish Red Spotted	53,554	992	1.85
Swedish Hornless	3,751	68	1.81
German lowland cattle	12,502	246	1.97
Finnish Ayrshire	57,082	750	1.31
New Zealand Jersey	87,926	901	1.02

The number of monozygous twins expressed as a percentage of the total twinnings varied between 0.05 and 0.28 in the above mentioned herds. The monozygotic twins are of great interest for scientific (experimental) reasons. The mono and dizygotic male twins could be distinguished not only on a morphological basis but by their sexual behaviour and the quality of the semen (Bielanski *et al.* 1966). In the uniparous laboratory animals it is relatively simple to establish lines and strains for twinning by inbreeding. The uniparous cattle species would need eight generations i.e. roughly 40 years to obtain 87% inbreeding, which is one of the reasons to disregard the possibility of increasing the number of twinnings by genetical means; in cattle breeding it is generally considered as an undesired way of reproduction (Peteu—Calotaiu 1967) or at least unsuitable for increasing beef production.

Based on the Hungarian breeding-register and progeny tests between 1956 and 1966 2.9% of the cows showed abortions. 38.1% of these was twin abortion (462,249 registered cows). In the state owned farms of 159,691 cows registered for breeding 75.4% calved, 4360 calved twins (3.63%) and there were only seven cases of triple twinnings. In 1969 among the 160,864 calvings of 219,912 registered cows in the state owned farms there occurred 5435 twinnings (3.37%) and 12 cases of triple twinning were recorded. The heterosexual twins showed an extremely high per cent of infertility (96%). According to the studies performed on a great number of animals in the state owned farms nearly half of the heifers born as twins showed infertility due to genital hypoplasia (intersexuality) which in turn led to an increase in cattle infertility.

Twinning in cattle has several other disadvantages: the gestation of twins to term imposes a great physiological burden on the dam which should be in good physical condition and of firm constitution. It is well known that twinning is always accomplished by laborious parturition (expedially if male twins are concerned) because of the presentation of the twins, mainly an adverse presentation, the simultaneous presentation of the limbs of the twins in the female genital tract, etc. In 40—48% of the parturitions the placenta or parts of it are retained (COMBERG—VELTEN 1962). Although the duration of gestation is usually shortened by one week as compared to single fetuses, the calving interval is prolonged by 6.4—6.5 days and the number of inseminations leading to conception always increases. The incidence of sterility is much higher after parturition of twins (25% higher on the average) because of the abrasions and the subsequent infection of the genital tract. As a consequence, the number of cows eliminated from breeding after twinning is 16 per cent higher than after single calving. The milk and milk fat production also decreases after twinning because the gestation means an abuse to the dam: the decrease in milk production may reach 46% (HENDY—BOWMAN 1970). In the case of twin fetuses the abortion rate is much higher than in those of single fetuses (PHILIPSEN 1956). The birth weight of twins is 20—30% lower, their vitality reduced, they require special care and are more susceptible to infections, which means that prenatal mortality is much higher among twins than among single calves; the prenatal mortality in a British-Friesian herd was 8 per cent and in a farm at the University of Nebraska 23 per cent higher among calves from twinnings. The incidence of stillborns is extremely high in twinnings: in a Holstein-Friesian herd at the Cornell University it was 7.6% in the single calvings and 34.4% in the twinnings; in an other Holstein-Friesian herd 9.65 and 22.7% and in a great Holstein-Friesian herd at Washington 12.5 and 38% of the calves were born dead in single and twin births, respectively. The same index was 5.3 and 23.7%, respectively, in a Holstein-Friesian herd in Ohio. In the Hungarian Spotted cattle the observation of 4360 and 5435 twinnings showed laborious parturition in 31.65 and 37.38%, among the calves 11.43 and 14.30%

were born dead or died within a few hours after birth. Up to weaning 21.44 and 30.35% of the rest were lost. Among the heifers born as twins 16.76 and 18.16% were eliminated for infertility (Table 2).

Table 2

Losses due to twinning in the Hungarian Spotted cattle

Year	Number of twinning	Number of calves	Calves dead		Laborious parturition		Calves lost up to weaning		Heifers eliminated for infertility	
			No.	%	No.	%	No.	%	No.	%
1966	4360	8728	998	11.43	1380	31.65	2121	27.44	731	16.76
1969	5435	10,882	1562	14.36	2032	37.38	2829	30.35	987	18.16

In the heterosexual twins the erythrocytes and the leucocytes show mosaicism as well as their haemoglobin and transferrin types; this can be used to learn their single or double ovulation origin which further contributes to the study of blood-groups. Sensitive and extensive serological studies may help, besides other methods, to predict if the heifer-twin is to be fecundated or not. It is of great economic importance because heifer-twins may be introduced to feeding earlier. Furthermore, twins are less suitable for feeding than singles. In Hungary studies have shown a 45.4% occurrence of sterility among heifer-twins (Hámori—Rónay 1965b) and in the red-dobrudsian cattle 30% of the heifer twins proved to be sterile (Peteu—Calotaiu, 1967). The heifers born as heterosexual twins are usually sterile (freemartinism) and 21.8% of them show poor food conversion, their weight gain is inferior to those born single, because of their unbalanced endocrine system and metabolism (Hámori—Rónay 1965a).

Venous-anastomoses in the placenta are more frequently and firmly developed if the fetuses are situated in the same horn of the uterus: blood-mixing was always demonstrated. On the other hand, with fetuses developing in the separate horns of the uterus the lack of anastomoses is possible and their genital tract may develop normally.

The dizygotic twin calves are immunologically tolerant against fraternal antigens.

Studies were performed in England to induce twin-gestations by hormone injections, but the twin-gestations had so many disadvantages that it was disregarded as a possible way for increasing beef production. The North-German Cattle Breeders Association imposes careful selection against twinning in cattle. Recently, attention was focused besides hormonal treatment and ovulation synchronization — on fertilized ova transplantation in order to increase the number of zygotes.

The chromosome picture of heterosexual (dizygotic) twins was studied by a number of research workers all over the world. BASROUR *et al.* (1970) demonstrated the presence of XX/XY sex-chromosome chimerism in quadruple twins, which was independent of the sexual phenotype and had a similar distribution both in males and females. On the basis of sexual chromosome chimerism the early prediction of sterility is possible in the heterosexual twins (KANAGAWA *et al.* 1965).

Interesting studies were performed in the bull-brothers of heterosexual twinnings of intersexual (freemartin) individuals concerning the fertility and chromosome pattern. The author found (1971) certain cow families with a high incidence of low conception rate and sterility, during 3—5 generations; in a twin-pair from one of these families XX/XY gonosomal chimerism was demonstrated with concomitant male pseudohermaphroditism and intersexuality in both calves; in an other twinning with an apparently normal 60 XY caryotype one of the calves showed male pseudohermaphroditism and intersexuality while the other had cryptorchism.

It was observed in the clinical, cytogenetical and seminal studies on phenotypically female but in fact intersexual twins that not only the intersexual calves with female appearance but their bull-brothers too show developmental abnormalities, semen of reduced fertility or even sterility in which an unbalanced hormonal system, metabolism and poor food conversion could be demonstrated. To sum up, the bulls from heterosexual twinning are inferior in twinnings, inferior in fertility, have worse food conversion, lighter slaughter weight than those from single birth.

Conclusions

Based on the above mentioned facts it can be concluded that twinning in cattle is a sophisticated breeding technique with several disadvantages. In order to put twinning into practice for increasing beef production further preliminary economic analysis and practical evaluation are necessary and it is not advised to introduce bull-offsprings from twinnings to breeding.

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ROLE OF LICHENS IN THE NITROGEN TURNOVER OF GRASSLAND COMMUNITIES ON SANDY AREAS

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Seasonal changes in the total N content of the xerophyte terricolous lichens *Cladonia magyarica*, *Cl. furcata*, *Cl. convoluta* and *Parmelia pokornyi*, as well as their role in the nitrogen turnover of the community were studied over two years (May 1970—April 1972) in grassland communities of sandy areas. Total N content in the lichen thalli — generally a value below 1 per cent — shows intensive seasonal changes. Differences between the individual species are in relation with the structure of the thallus. The share and role of the individual species in the nitrogen turnover of the plant community are determined by their mass conditions. Their role is more important in *Brometum tectorum* than in *Festucetum vaginatae*. The intensity of N turnover varies according to species and year.

Introduction

In the framework of investigations into the material turnover of grassland communities on sandy areas in the IBP PT section seasonal changes in the total N content of xerophyte terricolous lichens were studied. The objects set were: 1. to determine the changes in the total nitrogen content of lichen species and the lichen synusia of communities. 2. to reveal the role of lichens in the nitrogen turnover of grassland communities.

In earlier investigations the nitrogen content of lichens was found to range between fairly wide limits (0.4—7.5 per cent relative to the dry matter content). In general, the most frequent values are between 2 and 5 per cent (SMITH, 1960a). According to investigations made by SHAPIRO (1971) the total N content of terricolous lichens *(Cladonia alpestris, Cetraria islandica)* collected in Karelia and the neighbourhood of Leningrad was 0.4—0.6 per cent of their dry matter content. BASILEVICH—RODIN (1971) found the N content of xerophyte lichens in the steppe zone to be extremely low, not exceeding 1 per cent of the dry weight.

The investigations also included a study on the differences in nitrogen content and metabolic intensity between the gonidia and hyphae of lichen thalli. According to SOSA-BOURDOUIL (1944), in the thallus of *Usnea barbata* the N content is much higher in the gonidial layer than in the central medullar layer. Similar results were obtained in the course of detailed investigations by SMITH (1960b) who found the algal layer to have the most active part in the metabolism of the thallus. It had the highest nitrogen content as well as respiration and absorption rate.

It is the nitrogen compounds of soil, rain water and falling dust that may serve as nitrogen sources for the lichens. The fixation of atmospheric nitrogen only has a role in species whose gonidia consist of blue-green algae *(Cyanophycea)* (HENRIKSSON 1951, BOND—SCOTT 1955, SCOTT 1956). For the lichen species studied by us only soil and rain water can serve as nitrogen sources, since all four of them contain green algae *(Chlorophycea)*.

Material and Method

Our investigations were carried out on the IBP model area near Csévharaszt in the Danube-Tisza Midregion. For its botanical characterization see VERSEGHY—LÁNG (1971). From selected stands of the annual *(Brometum tectorum secaletosum)* and perennial open calciphilous *(Festucetum vaginatae danubiale)* grasslands of sandy soil four xerophyte lichen species important in the community were collected every month from May 1970 to April 1972. The species studied were: *Parmelia pokornyi* (Körb.) Szat., *Cladonia magyarica* Vain., *Cl. convoluta* Lam., and two varieties of *Cl. furcata:* var. *palmaea* (Ach.) Nyl. and var. *subrangiformis* (Sandst.) Abb. occurring in masses on the area concerned. From the carefully cleaned and selected material air-dry samples of 3 g quantity each were analysed in 2—3 replications per species and time of sample taking. The dry matter was digested in a mixture of concentrated H_2SO_4 and H_2O_2, with Se added as a catalyzer, according to Kjeldahl's method. From the dissolved material ammonia was released with NaOH, distilled through water vapour and absorbed by boric acid. Measuring was performed with 0.1 n HCl, in the presence of Groak's indicator. The results are expressed in mg % relative to the dry matter content of lichens. Calculations concerning the intensity of material turnover were made according to ROBERTSON (1957) and PRÉCSÉNYI (1971).

Results

Changes in the total N content of different lichen species. In the studied four lichen species the total N content is generally below 1 per cent — in agreement with the results obtained by BASILEVICH—RODIN (1971). The highest total N content was found in *Parmelia pokornyi*, while its value in the other three lichen species was lower. *Cl. furcata*, *Cl. convoluta* and *Cl. magyarica* show a declining order of succession, though there are no great differences between the individual species, especially in the case of *Cl. convoluta* and *Cl. magyarica* (Table 1). It is supposed to be in connection with the internal morphology of the lichen thallus, with the ratio of thickness of the medullar to the gonidial layer.

The ratio of the medullar to the gonidial layer in the species studied (as calculated on the basis of data given by VERSEGHY 1971) is shown in Table 2.

The gonidial layer is relatively the thickest in *Parmelia pokornyi;* the declining order of the ratio of medullar to gonidial layer in the species corresponds to their nitrogen contents.

Seasonal changes in the nitrogen content. No investigations have so far been made to determine the seasonal changes of the nitrogen content. Some data were presented by SMITH (1960a) who collected *Peltigera polydactyla* thalli in various periods of the year with the purpose of studying the nitrogen turnover in its discs. According to his measurements the total N content of the discs ranged between 3.55 and 4.54 per cent. Considerable fluctuations

Table 1

Average values of total nitrogen content as calculated from data obtained every month in the period of May 1970—April 1972 mg % dry matter of lichens

Species	Festucetum vaginatae, mg %	Brometum tectorum, mg %
Cl. magyarica	530—680	580—800
Cl. furcata	720—860	680—900
Cl. convoluta	500—700	600—800
P. pokornyi	600—900 and 800—1000	(700—1000)

Table 2

Ratio of medullar to gonidial layer

Species	Medullar layer, μ	Gonidial layer, μ	Ratio
P. pokornyi	148	92.5	1.6
Cl. furcata	99.5	37.0	2.1
Cl. magyarica Th.*	480	55.5	8.6
Cl. magyarica Pod.	261	97.0	2.7
Cl. Th. + Pod. mean	370.5	76.25	4.8
Cl. convoluta	185	27.7	6.6

* Th. = thallus
Pod. = podetium

occurred even within a short period; e.g., in November a difference of 300 mg% was found between the nitrogen contents of samples collected with a week's interval.

Remarkable seasonal fluctuations were found in the nitrogen contents of the studied xerophyte lichen species too. Species within the same community show different behaviour, and even the same species does not show identical trends in two different communities.

The total N content of the different species displays a monthly fluctuation, but seasonal changes can be observed as well. We tried to make these trends more expressed by the so-called "tendency curves" formed by a seasonal summarization (3 months average) of values measured every month.

The total nitrogen content of *Cladonia magyarica* shows different trends in the two communities. In *Festucetum vaginatae* maximum values were obtained fairly regularly in autumn in both years of the period of investigation, while in *Brometum tectorum* the maximum total nitrogen content appeared at

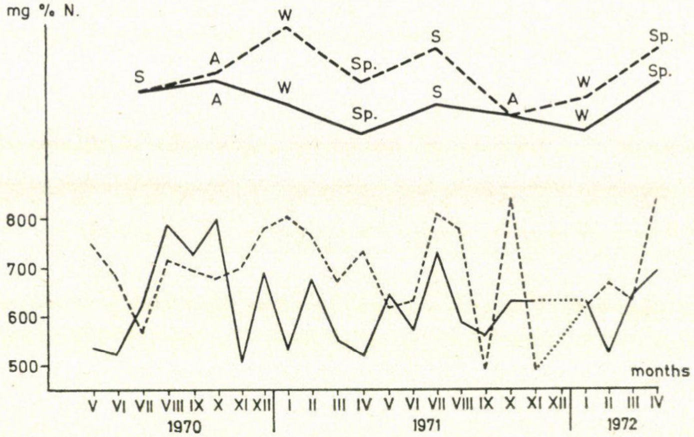

Fig. 1. Seasonal changes in the total nitrogen content of *Cladonia magyarica* in the grassland communities studied (—— *F. vaginatae,* ---- *Brometum tectorum;* S = summer, A = autumn, W = winter, Sp = spring)

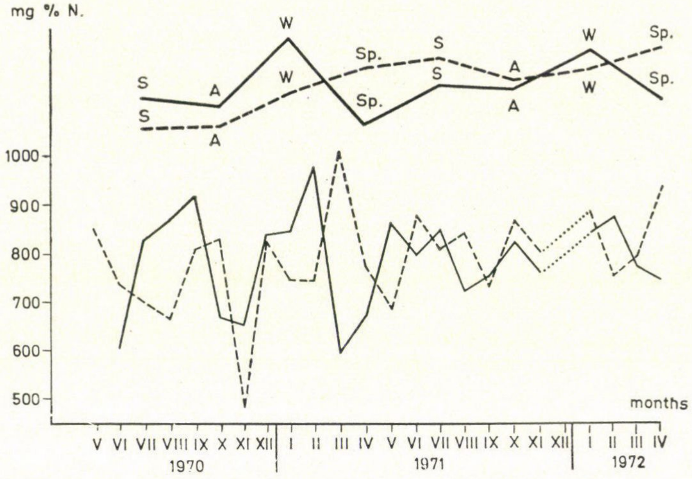

Fig. 2. Seasonal changes in the total nitrogen content of *Cladonia furcata* in the grassland communities studied (—— *F. vaginatae,* ---- *Brometum tectorum;* S = summer, A = autumn, W = sinter, Sp = spring)

different times (Fig. 1). In the latter community this value was somewhat higher than in the former one.

Changes in the total nitrogen content of *Cladonia furcata* show different trends in the perennial open calciphilous sandy grassland of *Festucetum vaginatae* and the annual *(Brometum tectorum)* grassland community of sandy soil. In *Festucetum vaginatae* the total N content of lichen increased in winter in both years of the period examined, while in *Brometum tectorum* the annual rhythm was indistinct (Fig. 2). The values of N content are similar in the two plant communities.

Seasonal changes in the N content of *Cladonia convoluta* differed in the two grassland communities only inasmuch as in the *Brometum tectorum* they showed a temporary decrease in the spring of 1971; otherwise the trend of the changes was the same. No similar values were obtained in the corresponding seasons of the two successive years. In the annual grassland of sandy soil *(Brometum)* the total N content of the lichen was on an average higher (Fig. 3).

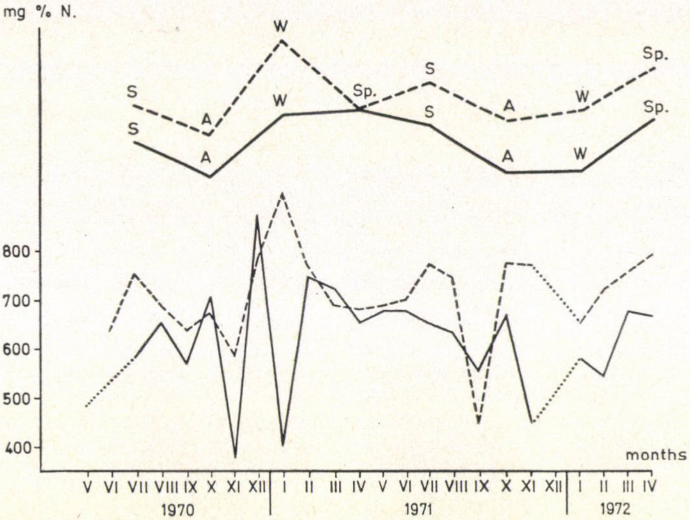

Fig. 3. Seasonal changes in the total nitrogen content of *Cladonia convoluta* in the grassland communities studied (—— *F. vaginatae*, ---- *Brometum tectorum;* S = summer, A = autumn, W = winter, Sp = spring)

Sufficient amounts of the lichen species *Parmelia pokornyi* could only be collected for analysis from the *Festucetum vaginatae* community. The total N content of this species was generally apparently higher than that of the other species analysed. The seasonal changes display a characteristic periodicity. There are periods when the total N content increases to such an extent that even the minima exceed 800 mg%. (The maximum value is 2600 mg%!) in comparison with the 700—100 mg% values of the following period (Table 1). Maximum N content generally occurs in the autumn—winter period (Fig. 4).

Total N content in the lichen synusia of the two plant communities. When comparing the total N content of lichen synusia in the two grassland communities we find great differences. The average values range between 650 and 850 mg%. Once in the period of investigation it rose above 1000 mg % in the perennial grassland community *(Parmelia pokornyi!)*, but the lowest values were also found in this community. In the first year the tendency of changes was found similar in the two communities, but in the second year it was different (Fig. 5). Seasonal changes in the total N content of lichen species

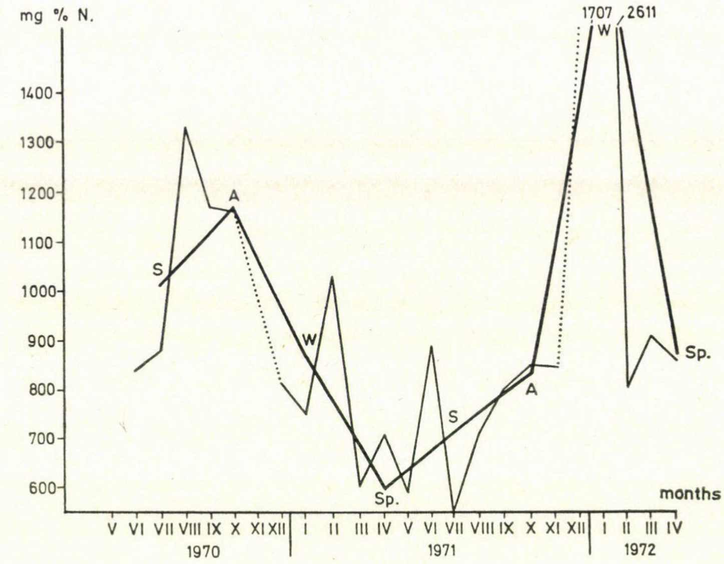

Fig. 4. Seasonal changes in the total nitrogen content of *Parmelia pokornyi* in the *Festucetum vaginatae* grassland community (—— *F. vaginatae*, ---- *Brometum tectorum;* S = summer, A = autumn, W = winter, Sp = spring)

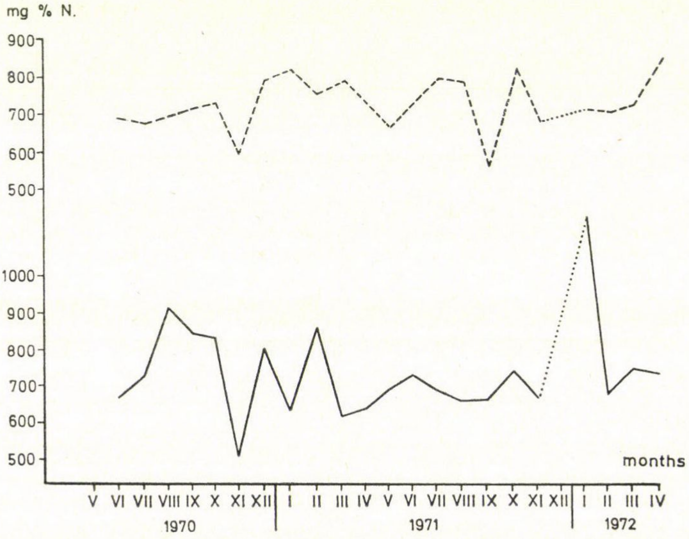

Fig. 5. Seasonal changes in the total N contents of lichen synusia in the grassland communities studied (—— *F. vaginatae*, ---- *Brometum tectorum*)

living in the same community showed the following trends in the two-year period: in *Festucetum vaginatae* it was only in certain periods that they were similar; e.g. in the spring of 1971 the total N content of 3 lichen species — *Cl. magyarica, Cl. furcata, Parmelia pokornyi* — showed a decreasing tendency compared to the previous period (see the tendency curves in Figs 1, 2 and 4). In *Brometum tectorum* changes in the total N content of *Cl. magyarica* and *Cl. convoluta* showed perfectly similar trends, and even in *Cl. furcata* it was only on one occasion — in the spring of 1971 — that any difference was observed (see the tendency curves of Figs 1—3).

The total N content and the lichen phytomass. From November 1970 production analyses were also conducted on the experiment area, as a continuation of investigations made in the previous years (VERSEGHY—LÁNG, 1971). In this way a picture was obtained of the amount of total nitrogen present in the phytomass of lichens on an area of 1 m^2 of each grassland community. The amount of N present in a certain lichen species per unit area reaches maximum when the phytomass production is the highest and the percentage N content of the species concerned shows a minimum value. This apparent contradiction might be explained by the fact that the total production of the species in question is at that time at its maximum, and the amount of N relative to the other components is lower. Thus the share and role of the individual species in the N turnover of the community may be determined by their mass conditions. E.g, the total amount of N/m^2 produced by *Cl. furcata*, the dominant lichen species of the annual *Brometum*, while ranging usually between 500 and 1000 mg, amounts to as much as 2400 mg/m^2 at the time of maximum production. In the species *Cl. magyarica* and *Cl. convoluta* occurring in much smaller masses values below 400 and 100 mg/m^2, respectively, were found in general. (Fig. 6). In the perennial grassland community of *Festucetum vaginatae* the mass conditions of *Cl. magyarica* and *Cl. furcata* were not essentially different, so their N contents per unit area did not considerably differ either: 150—500 mg/m^2 in *Cl. magyarica* and 50—400 mg/m^2 in *Cl. furcata*. The species *Cl. convoluta* and *Parmelia pokornyi* occur in essentially smaller masses; their N contents per 1 m^2 area are usually below 200 and 100 mg, respectively (Fig. 7).

Our data show, further, that in *Brometum tectorum* the total N content of the phytomass of lichens is higher than in the perennial *Festucetum vaginatae* due to the mass occurrence of *Cl. furcata*.

The intensity of nitrogen turnover. SMITH (1962) pointed out that lichens took up with relative easiness nitrogen compounds available in their habitat. In spite of this fact the incorporation of the nitrogen compounds taken up, as well as the protein decomposition of lichens are very slow processes. According to Smith the low rate of protein turnover is a general feature of lichens and is in connection with their slow growth rates and longevity.

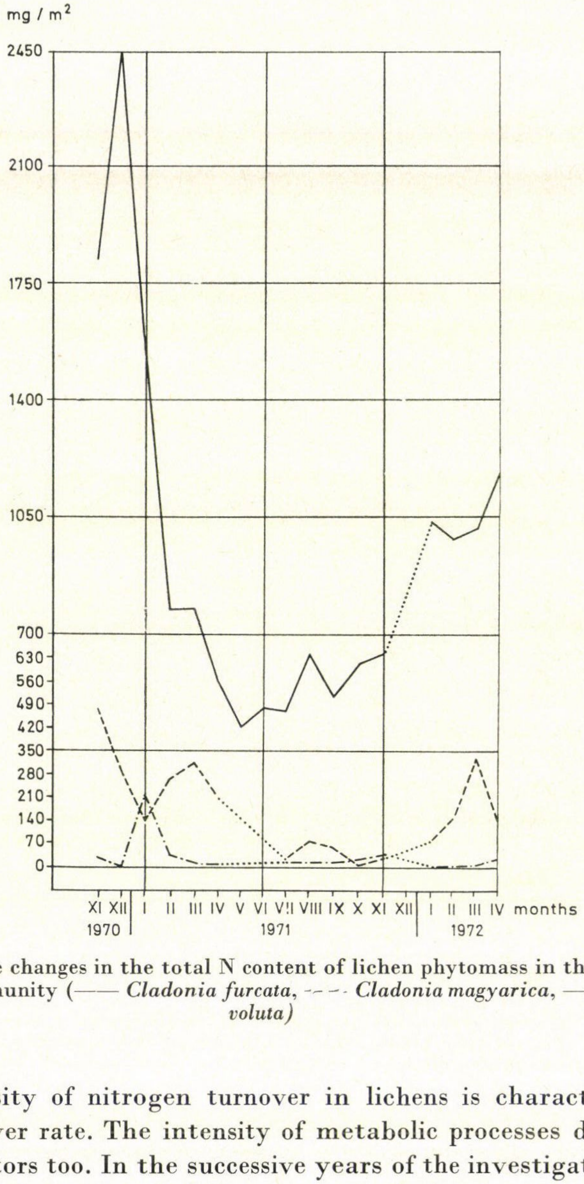

Fig. 6. Quantitative changes in the total N content of lichen phytomass in the *Brometum tectorum* grassland community (—— *Cladonia furcata,* - - - *Cladonia magyarica,* —.— *Cladonia convoluta)*

The intensity of nitrogen turnover in lichens is characterized by the values of turnover rate. The intensity of metabolic processes depends on environmental factors too. In the successive years of the investigation period the environmental factors showed different trends. As for the climatic conditions, the most outstanding differences were found in the amount and distribution of precipitation. In the first year of investigation (April 1970 – March 1971) the amount of precipitation on the area studied was 597 mm with maxima in June, August and January. In the second year (April 1971 – March 1972) the amount of precipitation was as low as 376 mm; the period between May and September was especially remarkable for its very low amount of precipitation. The development of plants in the grassland community as well as their conditions of competition changed parallel with the above circumstances.

The turnover rate and turnover time of nitrogen in the four lichen species studied vary according to year and species. In *Festucetum vaginatae* the turnover rate — i.e. the intensity of nitrogen metabolism — was lower in both years in *Cladonia magyarica* (0.35, 0.28),* *Cl. furcata* (0.37, 0.17) and *Cl. convoluta* (0.56, 0.33) than in *Parmelia pokornyi* (0.54, 0.79).

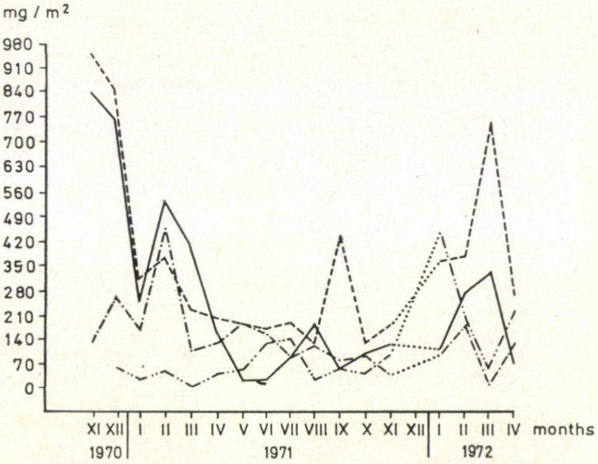

Fig. 7. Quantitative changes in the total N content of lichen phytomass in the *Festucetum vaginatae* grassland community (—— *Cladonia furcata*, ---- *Cladonia magyarica*, —·— *Cladonia convoluta*, —··— *Parmelia pokornyi*)

Turnover rates were lower — with the exception of *Parmelia pokornyi* — in the second year than in the previous one, that is, the intensity of the nitrogen turnover decreased under the influence of the unfavourable environmental conditions. The turnover time increased accordingly from 2.5 to 3.6 years in *Cl. magyarica*, from 2.7 to 5.9 years in *Cl. furcata*, from 1.8 to 3.0 years in *Cl. convoluta*, while in *Parmelia pokornyi* decreased from 1.8 to 1.3 years.

Especially great differences were shown in the two successive years by the nitrogen turnover of *Cl. furcata;* its intensity decreased by 50 per cent in the less favourable year (the turnover time redoubled). This lichen species showed a totally similar behaviour in the annual community of *Brometum tectorum*, where its turnover rate decreased from 0.51 to 0.25.

When in the perennial grassland community of sandy soil *(Festucetum vaginatae)* we study the nitrogen turnover of the lichen synusium as a whole

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* The first figure characterizes the period between May 1970 and April 1971, the second figure the period between May 1971 and April 1972.

we find the turnover rate and time to be identical in the two years (0.43 and 2,3 years) in spite of the fact that the nitrogen turnover of the individual species — as we could see — is different. From the point of view of nitrogen turnover the behaviour of the lichen synusium differs from that of the individual species composing the synusium.

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EFFECT OF 6-METHYLURACYL AND 2-CHLORO-ETHYL-PHOSPHONIC ACID ON THE FRUCTIFICATION AND CROP OF VARIOUS STRAINS OF CHAMPIGNON AGARICUS BISPORUS (LANGE) SINGER

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The authors conducted experiments to study the effect of 6-methyluracyl and 2-chloroethyl-phosphonic acid on the yield and fructification of various strains of champignon *Agaricus bisporus* (Lange) Singer under small-scale conditions. The examined strains were partly the most often cultivated ones (earlier; Pc-17: today D-13, F-1), partly those not widely introduced in Hungary as yet (SO-9, Z-3). It was found that both active agents — when added to the compost — considerably accelerated the appearance of fruit bodies compared to the untreated controls. Under the influence of the treatments the fructification periods became more even, especially in the case of the strains SO-9 and Z-3. The active substances increased the yield averages of the strains D-13, F-1, SO-9 and Z-3 to a lower, while that of the strain Pc-17 to a considerable extent. The yield averages of the examined strains showed maximum values at different concentrations: the stimulation — though sometimes attaining 35—50 per cent — was 15—30 per cent on an average. 2-cloroethyl-phosphonic acid and the cytokinin-type 6-methyluracyl-influenced fructification in strains showing different morphological and physiological characteristics. This fact seems to prove the growth-regulating effect of the compounds used in the process of mushroom production too. The results of the experiments seem to be utilizable — after further investigations — under the large-scale conditions of mushroom production as well. Their physiological implications may contribute to studies on the mode of action of fructification, as well as of 6-methyluracyl and 2-chloroethyl-phosphonic acid.

Introduction

Nowadays champignon production is one of the largest-scale branches of agriculture. It supplies the market with valuable nutritive material relatively irrespective of the season. In the mushroom producing countries all over the world great financial and intellectual efforts are made to increase the yield of champignon production. Full mechanization of the work processes, individual phases of production, establishment of controlled condition buildings, introduction of new production technologies, breeding by variety selection, preparation of the compost and replacement of the horse manure are the main fields where the increase of yield averages can be ensured.

It was in the course of studying the physiological processes of plants that the possibility arose of using growth regulators in plant production. The cytokinins which exercise a manifold and not fully explained effect on metabolism are of basic importance in the processes of growth and development (**Helgeson** 1968, **Phillips** 1971). Our work is based on experiments pointing out that under the influence of certain bioactive substances sterile mycelium cultures of champignon and other basidiomycetes changes were found in growth and other induces of metabolism too (**Vetter—Maróti** 1971, **Szabó** *et al.* 1972). The aim of our experiment was to study the effect of compounds found the most active (6-methyluracyl and 2-chloroethyl-phosphonic acid) on the production periods and yields of five champignon strains under small-scale conditions.

Material and Method

In our experiments we studied various strains of the cultivated mushroom *Agaricus bisporus* (Lange) Singer. These more or less differ from one another regarding their morphology and production biology. Strains included in the experiments were D-13, F-1, Pc-17, SO-9 and Z-3. These strains belong to different forms of *Agaricus bisporus* var. *albidus* (Lange) Singer (**Singer** 1961). D-13 is a monosporal inbred strain originating from Denmark, with white or slightly creamy colour, flattened pileus and cylindrical stem: it can be ranked with f. *langei* Bohus. The pilei of fruit bodies in the inbred strain of F-1 are more or less sunken, concave or flat. The surface of the cream-coloured skin of the pileus may be fibrous or lamellar. Its stem is short and conic, therefore this strain belongs to F. *conicopus* Bohus. Pc-17 has been developed from a wild strain: it is of a creamy colour similar to that of the former strain, but its pileus is convex or flat at the most, and the skin of the pileus is more intensively scaled, tomentose and broken. The stem is thick, cylindrical. It can be ranked with f. *langei* Bohus (**Uzonyi—Bohus** 1962). Z-3 is closer to F-1 while SO-9 to D-13.

The experiments and the evaluation were carried out at the Department of Botany and in the Botanical Garden of the University of Horticulture as well as at the Department of Botany of the University of Veterinary Sciences. A plastic bag-production technology well proved abroad and in the Duna Co-operative Farm was modified and adapted to our small-scale experiments, as already described in an earlier paper (**Rimóczi—Vetter** 1973). The effects of 2-chloroethyl-phosphonic acid and a pyrimidin derivative; 6-methyluracyl were studied. The concentrations used were; 2.5, 5.0, 20 ml/1.5 kg compost of 1000 ppm aqueous solution of 6-methyluracyl and 100 ppm aqueous solution of 2-chloroethyl-phosphonic acid. In the experiments the treatments were performed with six simultaneously started replications per strain. In the course of the experiment we followed with attention the time of appearance of the fruit bodies, the periodicity of production waves, and weighed the amount of yield (kg/q compost). In each case only first-class fruit bodies possessing intact velum were picked. Data on the production waves are presented in detail, without averaging, totalled according to treatment: the yields on the basis of our earlier work with the means (as a percentage of the control as well) and the error (**Vetter—Maróti** 1971).

Results

In our production experiments the effect of various quantities of 6-methyluracyl and 2-chloroethyl-phosphonic acid added to the compost on the period of fructification and amount of yield was studied with five strains of champignon. Following the casing, the mycelium interwove the peaty sandstone powder in a very short time. This process could be followed especially well at the edges of the plastic bags. The fruit body fundaments mostly appeared in groups close to the edges of the plastic bags 1—1.5 mm deep under the casing and rose to the surface with 2—5 mm diameters. All this took place in the control bags in the third and fourth week after planting, depending on the strain. Thus in strains D-13 and SO-9 the first ripe mushrooms appeared on the 32nd, while in strain F-1 on the 33rd day. With strain Pc-17, ripe fruit bodies could only be found on the 35th, and in the case of Z-2, on the 37th day after planting. According to our observations, the development of strain Pc-17 was relatively slow compared to the other strains. In this respect the strain Z-3 is closer to Pc-17. As to its rapid growth and development the third cream-coloured strain — F-1 — is similar to the white D-13 and SO-9 strains.

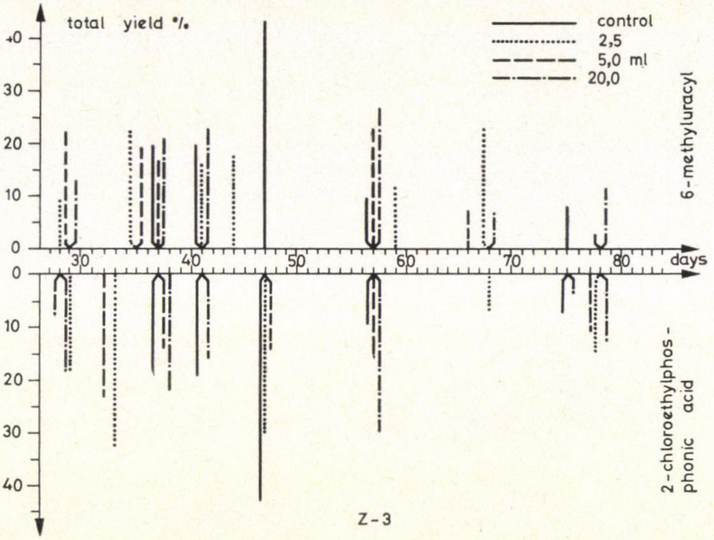

Fig. 1. Trend of the fructification period of the champignon strain Z-3 on compost treated with 6-methyluracyl and 2-chloroethyl-phosphonic acid. Abscissa: days from plantation; ordinate: daily amounts of yield as a percentage of total yield

In general, fructification started earlier on the treated bags than on the controls. The time the fruit bodies appeared depended on the strain and on the concentration of the active substance used. The highest effect was found in strain Z-3, where ripe fruit bodies were picked as early as on the 28th and 29th day after planting with all concentrations of the active substance, while in the control only the first fruit body fundaments appeared at that time (Fig. 1). In strain Pc-17 the first fruit bodies were picked seven days earlier than in the control (Fig. 2). In strain F-1 the first fruit bodies were picked on the 25th and 26th day, while on normal compost the same strain only gave mature fruit bodies on the 34th day (Fig. 3). The white fruit bodies of strains SO-9 and D-13 appeared on the enriched compost on the 26th day alike, 5—6 days earlier than in the controls of the same strains (Figs 4 and 5).

During the two-month period of cultivation the number of production waves was generally six. Application of 6-methyluracyl and 2-chloroethyl-

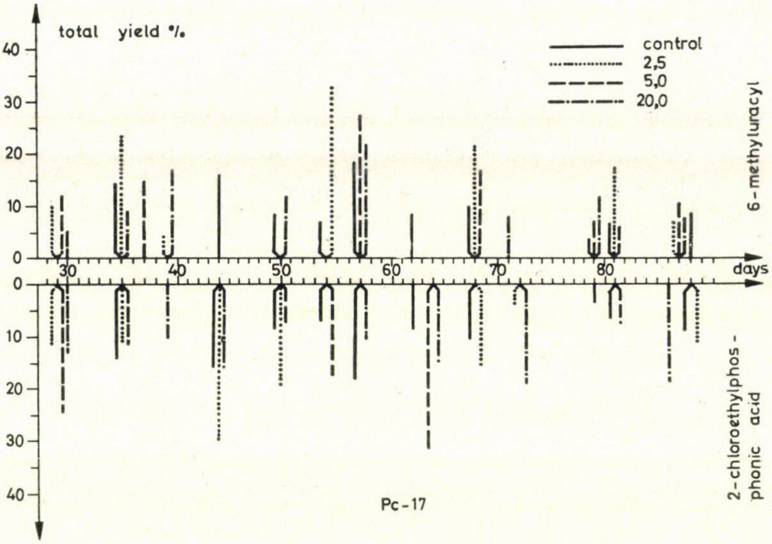

Fig. 2. Fructification period of the champignon strain Pc-17 on compost treated with 6-methyluracyl and 2-chloroethyl-phosphonic acid. Abscissa: number of days from plantation: ordinate: daily amounts of yield as a percentage of total yield

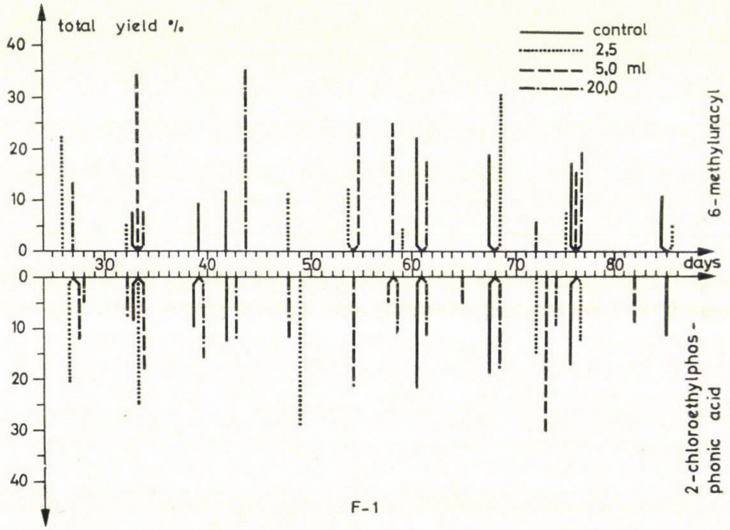

Fig. 3. Effect of treatments with 6-methyluracyl and 2-chloroethyl-phosphonic acid on the fructification period of the champignon strain F-1. Abscissa: days from plantation; ordinate: daily amounts of yield as a percentage of total yield

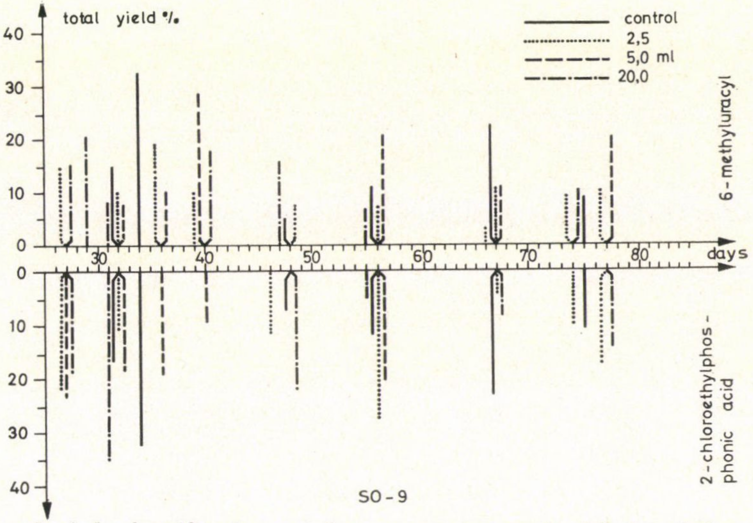

Fig. 4. Trend of the fructification period on compost treated with 6-methyluracyl and 2-chloroethyl-phosphonic acid in the case of the champignon strain SO-9. Abscissa: days from plantation; ordinate: daily amount of yield as a percentage of total yield

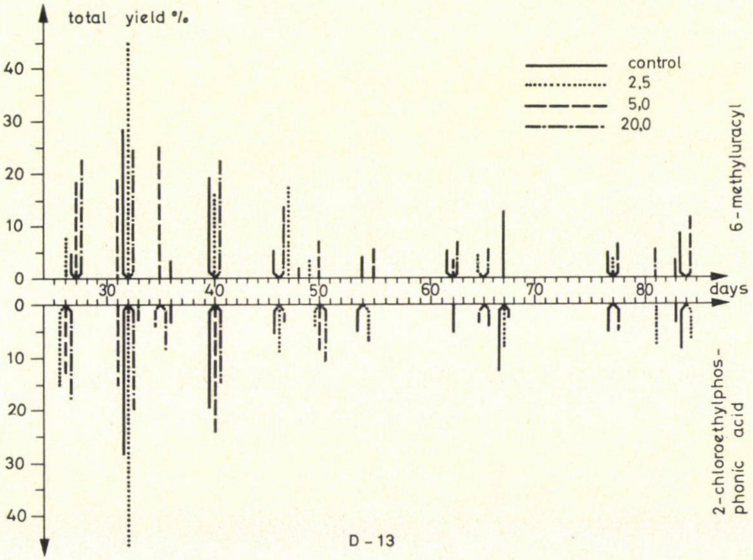

Fig. 5. Trend of the fructification period of the champignon strain D-13 on a compost treated with 6-methyluracyl and 2-chloroethyl-phosphonic acid. Abscissa: days from plantation; ordinate: daily amounts of yield as a percentage of total yield

phosphonic acid modified the production waves observed in the controls but partly. It is characteristic that the first two or three production waves give 70—80 per cent of the total yield. They are closer to each other in time, while between the last waves intervals of even 8—10 days may occur. Both active substances make the production waves more even. This can be best seen in strains SO-9 and Z-3 (Figs 1 and 4). The period of cultivation is not completed earlier under the influence of the treatments (except in some cases in strain F-1).

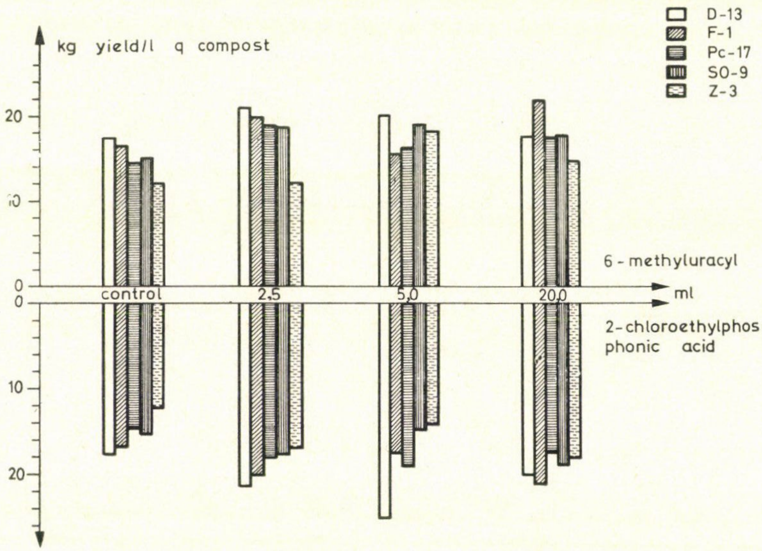

Fig. 6. Trend of yield (kg/q compost) in the champignon strains D-13, F-1, Pc-17, SO-9 and Z-3 on a compost treated with various concentrations of 6-methyluracyl and 2-chloroethylphosphonic acid. The presented values of ml represent the quantities per 1.5 kg compost of a 1000 ppm stock solution of 6-methyluracyl and 100 ppm stock solution of 2-chloroethylphosphonic acid

On a compost which does not contain any active substance, strain D-13 shows the highest yield average (17.6 kg/q compost). This justifies — among others — the fact that D-13 is the most important strain in cultivation at present. The yield average of F-1 is 16.7 kg/q compost, while that of SO-9 15.3 kg/q compost. In our experiment the lowest yield was obtained with strain Z-3 (12.2 kg/q compost) (Table and Fig. 6). The effect of 6-methyl-uracyl and 2-chloroethyl-phosphonic acid was felt in the increased yield averages too. The yield average of D-13 was increased by all concentrations of 2-chloroethylphosphonic acid. Treatment with 5 ml solution of 100 ppm was especially efficient, increasing the yield by 42 per cent. This 25 kg/q compost yield average was the highest production result in our experiment series. Enrichment with 6-methyluracyl was successful only at lower concentrations (+ 21 per cent),

Table 1

Effect of 6-methyluracyl and 2-chloroethyl-phosphonic acid on the yield averages of Agaricus bisporus (Lange) Singer strains. $\bar{x}$ *= yield average (kg/q compost); s = error; % = yield average as a percentage of the untreated control. The given concentrations mean the ml amounts of a 1000 ppm basic solution of* **6**-*methyluracyl and 100 ppm basic solution of 2-chloroethyl–phosphonic acid per 1.5 kg compost*

Champignon strain		Control	6-methyluracyl (in ml-s of the basic solution)			2-chloroethyl-phosphonic acid (in ml-s of the basic solution		
			2.5	5.0	20.0	2.5	5.0	20.0
D-13	$\bar{x}$	17.6	21.2	20.2	17.6	21.4	25.0	20.0
	s	2.8	2.2	2.3	1.7	2.1	3.3	4.0
	%	100	121	115	100	122	142	114
F-1	$\bar{x}$	16.7	20.0	15.6	22.0	16.8	17.4	21.4
	s	3.0	2.2	1.6	0.7	2.0	1.2	3.0
	%	100	121	93	132	100	105	127
Pc-17	$\bar{x}$	14.6	19.0	16.3	17.5	18.0	19.2	17.3
	s	2.1	3.0	1.7	2.6	2.7	2.3	2.3
	%	100	130	112	120	123	131	118
SO-9	x	15.3	18.8	19.2	17.8	17.6	14.7	18.8
	s	2.3	1.4	2.8	2.6	3.4	4.9	2.7
	%	100	122	126	116	115	94	120
Z-3	$\bar{x}$	12.2	14.3	18.4	16.6	16.8	14.0	18.0
	s	3.1	1.8	2.6	3.7	2.8	1.3	2.9
	%	100	117	152	137	137	115	147

but resulted in an earlier appearance of fruit bodies at all concentrations. 6-methyluracyl, when applied at concentrations of 2.5 and 20 ml, increased the 16.7 kg/q compost yield average of strain F-1 by 21 and 32 per cent, respectively, while the 5 ml treatment — just like 2.5 and 5 ml of 2-chloroethyl-phosphonic acid — proved ineffective. At the same time, these concentrations had a favourable, equalizing effect on the production period and the first fruit bodies could be picked almost a week earlier.

The yield average of strain Pc-17 is 14.5 kg/q compost. Both active substances at all concentrations increased this value to the nearly 18 kg yield average obtained with the strain D-13. SO-9 with its white pileus and 15.3 kg/q compost is similar to D-13. This amount of yield was increased almost in the same measure by all concentrations of 6-methyluracyl to nearly 19 kg/q compost. At the same time 5 ml of 2-chloroethyl-phosphonic acid did not raise this yield, although under its influence the culture turned into bearing 5 days earlier. The strain Z-3 can be characterized by an average yield of 12.2 kg/q compost. This value can be increased by 47 per cent with 2-chloroethyl-phosphonic acid, and by 52 per cent with 6-methyluracyl applied.

In our experiment series we studied the effects of a cytokinin-type pyrimidin derivative: 6-methyluracyl, and of another — similarly bioactive — substance: 2-chloroethyl-phosphonic acid on fructification in champignon strains, as reflected by the yields. According to the evidence of earlier experiments performed with flowering plants, 6-methyluracyl — like the natural cytokinins — proved to exercise an inhibitory effect on senescene in the case of *Phaseolus vulgaris;* it stimulated the incorporation of labelled nucleic acid bases (POZSÁR 1972). It seems probable that this compound acts through the nucleic acid and protein metabolism, although the details are not clear yet (POZSÁR—MATOLCSY 1968). The action spectre of the other compound used is wider and at the same time more contradictory. While stimulating the germination, it inhibited the growth of the seedlings (CHATTERJI *et al.* 1971), and decreased — on the other hand — the inhibition of germination caused by auxin (SANKHLA—SANKHLA 1972). The action mechanism of 2-chloroethyl-phosphonic acid may be related with the ethylene, as it has been demonstrated in vitro that ethylene is released from 2-chloroethyl-phosphonic acid. It can be assumed that the same may occur in the living organism as well (KNYPL 1971). According to the most recent data the ethylene — though generally acting as an inhibitor — may stimulate certain processes (SUGE 1971).

In our experiment series all treatments of the different champignon strains were evaluated from the point of view of components (dry matter, RNA, DNA and protein content) as well. In the course of the analyses an unambiguous correlation was found between the increased yield average attained with the treatments (stimulated growth) and the high RNA, DNA and protein content of fruit bodies (RIMÓCZI—VETTER 1973). According to the data of the present paper both regulations accelerated the fructification and made it more even in all strains examined.

The question of how the two compounds accelerated the fructificaton would be very difficult to answer, all the more so because this process depends on many, at present unknown factors. As regards tendency, the two different structures and probably differently acting compounds had about the same influence on fructification. It can be supposed that the stimulation either affected the fructification directly or the pathway starting with the introduction of the mycelium into the soil and leading to the fructification indirectly.

Our results — besides their utilization in mushroom production — may be of use in studying the substance regulating fructification on one hand, and in general growth and development on the other.

Acknowledgements

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DEVELOPMENT OF RAPHIDE IDIOBLASTS IN THE AERIAL ROOT OF MONSTERA DELICIOSA LIEBM.

By

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The authors studied the development and differentiation of raphide idioblasts in the aerial root of *Monstera deliciosa.* They found these cells to be reproduced by inequal division and have large nuclei and active cytoplasms. The crystalliferous cells are arranged linearly in several rows, and the idioblasts placed at some distance above each other may touch, due to their growing more rapidly than the surroundings.

Introduction

In the course of earlier studies (KOVÁCS—RAKOVÁN—SZUJKÓ-LACZA 1972, RAKOVÁN—KOVÁCS—SZUJKÓ-LACZA 1973) we dealt with the development of calcium oxalate raphides found in the aerial root of *Monstera deliciosa.* As a continuation of the study, we considered it necessary to follow the formation and differentation of the idioblast itself. The present paper gives an account of the light- and electron-microscope observations connected with this question.

It is known that the development of many special plant cells (e.g. stoma, root-hair, glandular hair, trichosclereid) begins with unequal cell division. It is supposedly the case with the crystalliferous idioblasts too (e.g. KÜSTER 1956, FOSTER 1956). FRANK (1967), on the other hand, observed equal division. No such publication has been found so far as demonstrating by original photos that the initials of the crystalliferous idioblasts are produced either by equal or by unequal cell division.

The young idioblasts can be distinguished from the surrounding cells by their large nuclei and sudden growth even in the meristematic tissues (KOWALEWITZ 1956). The multiplied size of the nucleus can be the consequence of endomitosis, though RENNER *et al.* (1952) and KOWALEWITZ (1956) found crystalliferous cells with two nuclei. SCHÖTZ *et al.* (1970) point out, on the other hand, that the idioblasts can divide too, and it is in this way that the twin cells observed by them in the leaf of *Oenothera* are produced.

The general opinion that the cytoplasm and nucleus of idioblasts containing crystals degenerate more rapidly than those of the surrounding cells was disputed by MOLLENHAUER—LARSON (1966) who brought the secretion activity of the cytoplasm into connection with the crystal formation.

In certain cases the raphide idioblasts may be 20—40 times as large as the surroundings cells, and according to ARNOTT (1962) can be found in the root tip in linear rows. On the basis of SCHOCK-BODMER—HUBER (1951), KOWALEWITZ (1956) supposes that the idioblasts develop by an apical growth similar to that of the phloem fiber.

Material and Methods

In the light-microscope studies the apex of a growing aerial root of 5—7 mm diameter as well as 2 cm of the zone close to the apex were used. The material was fixed in Bouen and embedded in paraffin; then with a microtome 10—12 micron thick longitudinal sections were made and stained with haematoxyline.

In the electron-microscope studies the apex of the aerial root and the periblem of an about 1/2 cm zone close to the apex were used. The material was fixed in 2 per cent $KMnO_4$, dehydrated with alcohol, embedded in durcupan, then contrasted with uranyl acetate and lead citrate. The electron micrographs were taken with a KEM-I type GDR make electron microscope.

Results

In the aerial root of *Monstera deliciosa* Liebm. the raphide idioblasts are found in the periblem then in the outer half of the cortex. The initials of the idioblasts are produced first of all in the periblem near the apex by unequal division. The unequal division can be recognized morphologically too, and the smaller of the two cells produced — the one detached from a corner of the isodiametric mother cell — will be the initial of the idioblasts (Fig. 1).

However, the initials of idioblasts develop not only in the immediate vicinity of the apex, but also at a distance of 5—7 mm from it, from the outer subdermatogenous, sometimes vacuolizing cells of the periblem, which remain meristemic for a long time (Fig. 2).

In the idioblast initials — as soon as they reach the size of the surrounding cells — the initials of raphides can be seen with an electron microscope (Fig. 3), and when they grow two- or threefold even with a light microscope (Fig. 4). There is no suitable photo available to prove that it is the smaller daughter cell of the unequal division that becomes idioblast initial, but we were convinced of this being the fact in our investigations.

The young idioblasts are characterized by large nuclei (Fig. 5). At the beginning the crystals are arranged more or less radially round the large nucleus in the cytoplasm (Fig. 4), however, as soon as the cell continues growing, the crystals increase in number and take a position along the longitudinal axis of the idioblast (Fig. 5).

In connection with the development of the idioblast we observed that the zone bending in accordance with the dermatogenous curve was in the periblem, at the height of the central initial cells — in a radial longitudinal section —, and after a number of equal divisions every third or fourth of these cells divided unequally. The latter are the initials of new idioblasts. The alternation

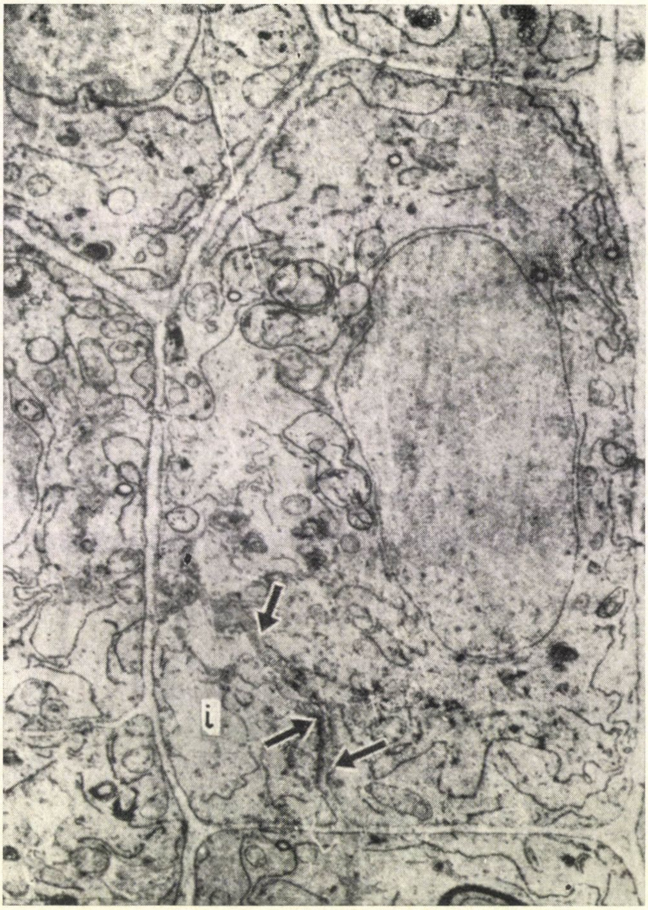

Fig. 1. Unequally divided periblem cell, the smaller daughter cell is the raphide-idioblast initial (i): the arrows indicate the developed new cell-wall. EM: 5500 ×

of equal and unequal divisions results in both the longitudinally placed idioblasts and those arranged in parallel rows (Fig. 7). Young idioblasts developing newly in the subdermatogenous layer of the periblem found at some distance from the apex become initial cells of new linear crystalliferous rows (Fig. 6).

The rapid growth of the idioblasts begins with the sudden enlargement of the nucleus which may become 3—5 times as large as the nuclei of the surrounding cells. The cell wall — especially at the apex of the cell — becomes much thicker; the idioblasts sharpened at the tip may enter between the surrounding cells and force them apart (Fig. 8). During their growth the idioblasts forming longitudinal rows may touch (Fig. 9), or may even continue to grow for a while side by side (Figs 10, 11). We also observed young idioblasts set close to one another, however, they were twin idioblasts, that is, originating from

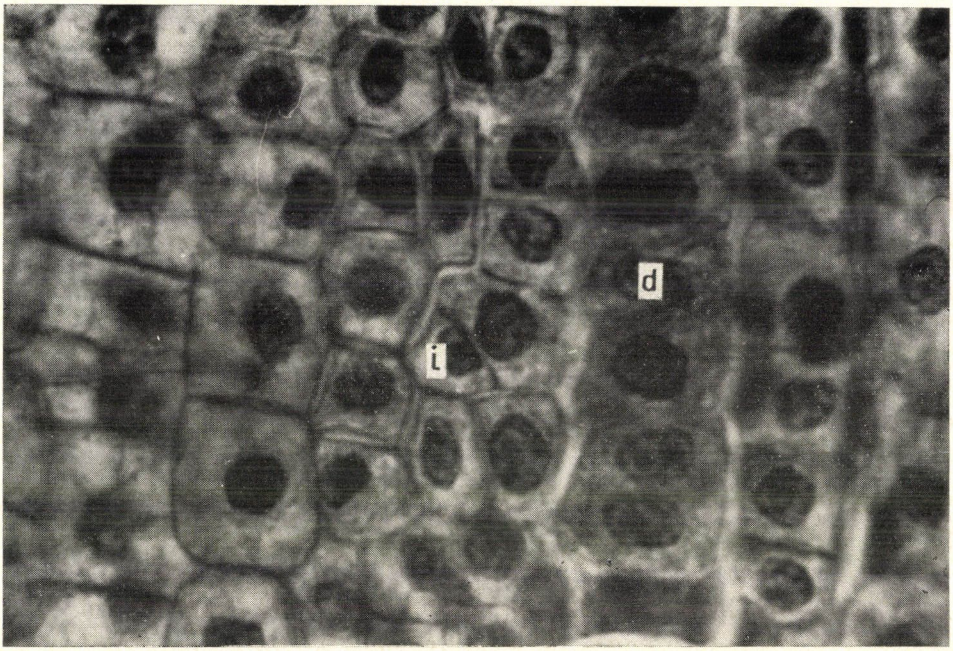

Fig. 2. Unequally divided cell in the cell row next to the dermatogen, the smaller daughter cell is the raphide-idioblast initial (i). Part of a longitudinal section at a few mm distance from the central group of the root-tip initial. LM: 1000 ×

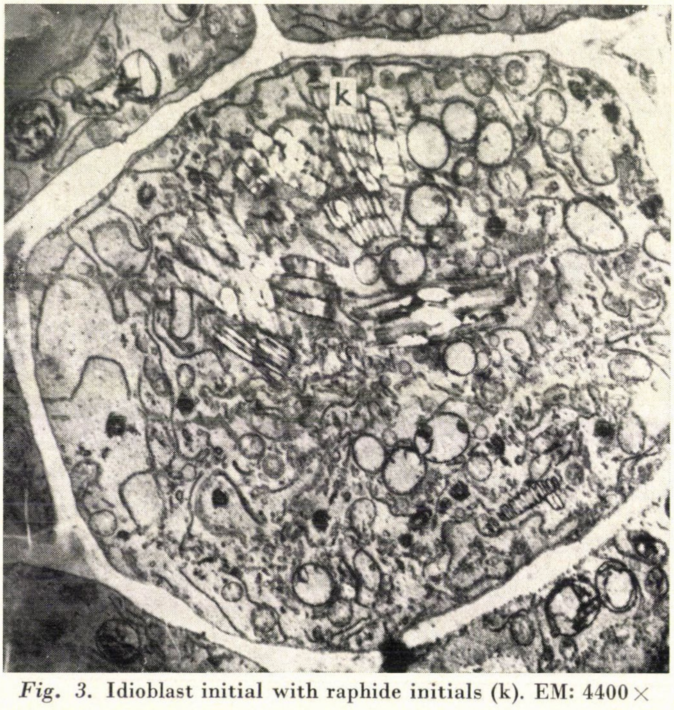

Fig. 3. Idioblast initial with raphide initials (k). EM: 4400 ×

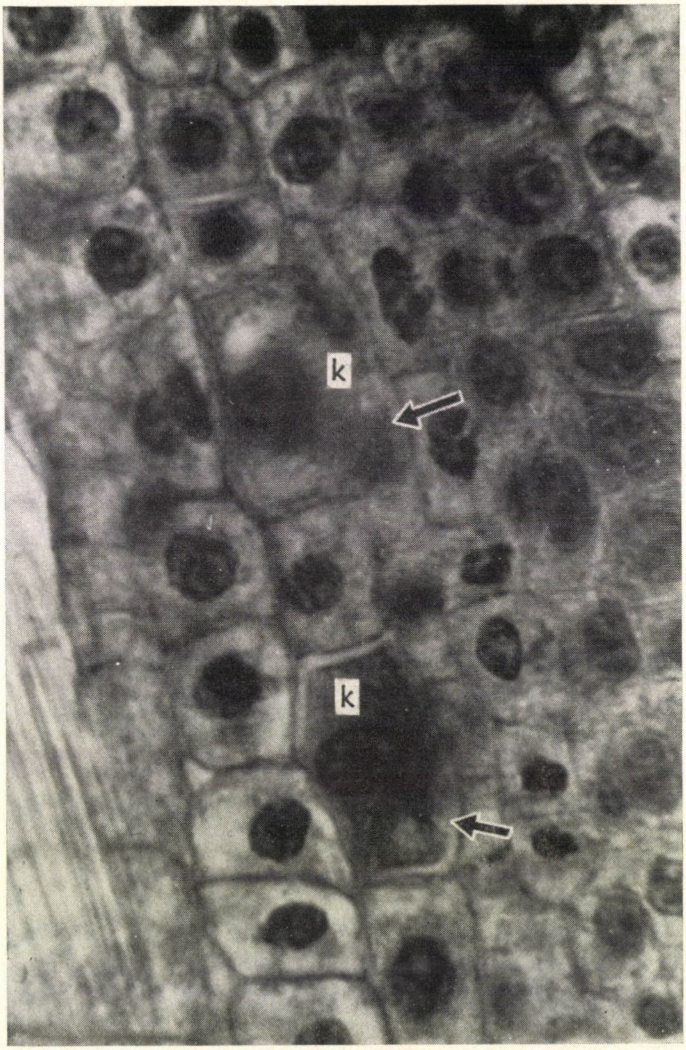

Fig. 4. Idioblasts increased twofold (←) with radially arranged raphides (k). Part of a longitudinal section from the periblem. LM; 1000 ×

the equal division of the idioblast initial (Fig. 12). There is no close cytoplasmic connection between the crystalliferous cells and their surroundings, as also indicated by the absence of plasmodesms (Figs 13a, 13b). At the same time, the absence of the plasmodesms supports our hypothesis of idioblasts growing more rapidly than their surroundings.

The crystalliferous cells may even increase two- or threefold in width compared to the adjacent cells. In a fully developed state their length may

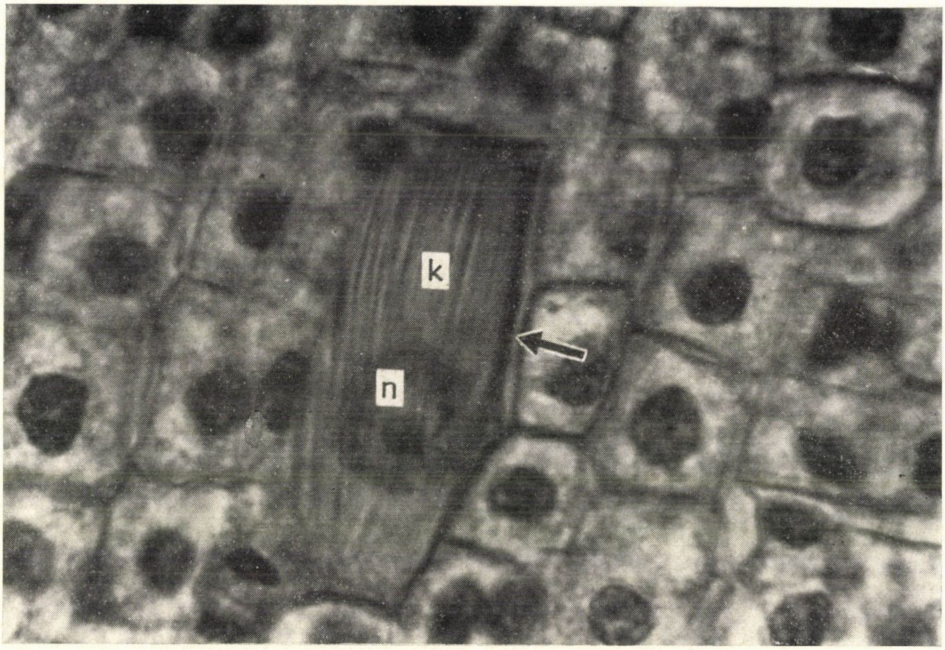

Fig. 5. Young idioblast (←) with a giant nucleus (n) and parallelly oriented raphides (k). LM: 1000×

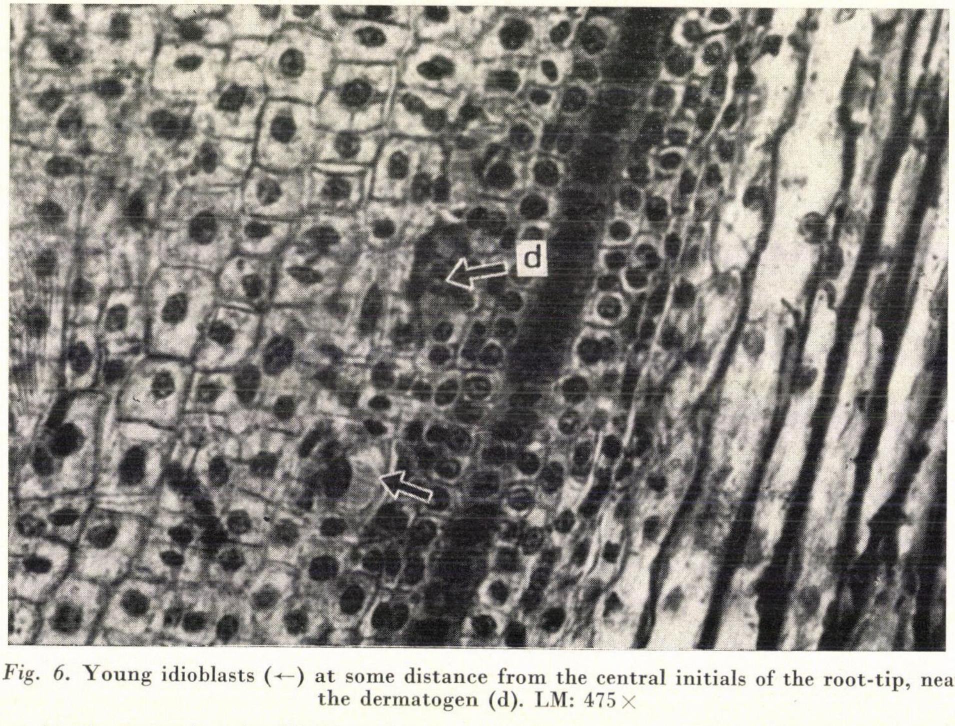

Fig. 6. Young idioblasts (←) at some distance from the central initials of the root-tip, near the dermatogen (d). LM: 475×

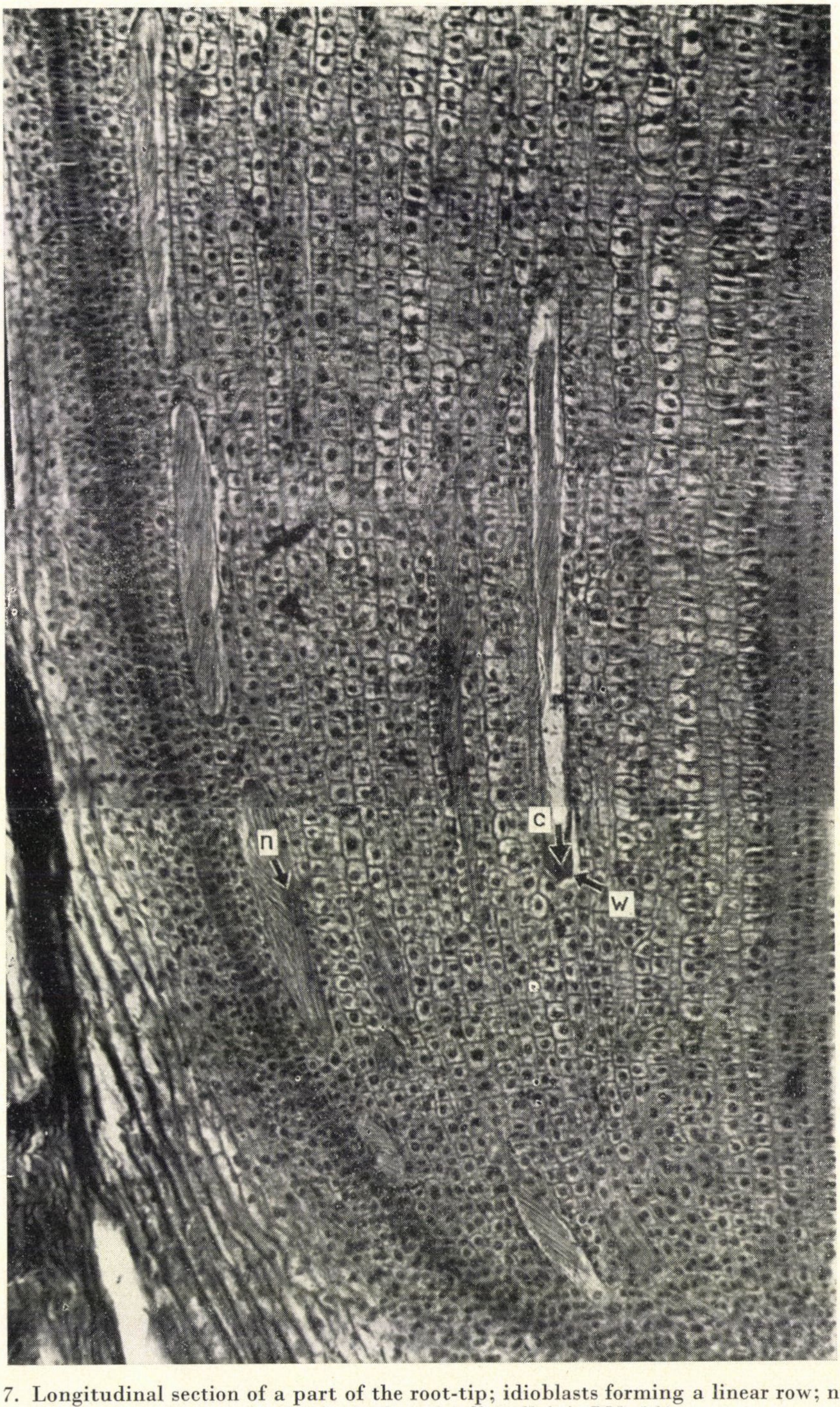

Fig. 7. Longitudinal section of a part of the root-tip; idioblasts forming a linear row; nucleus (n), cytoplasm (c), thickened cell-wall (w). LM: 160 ×

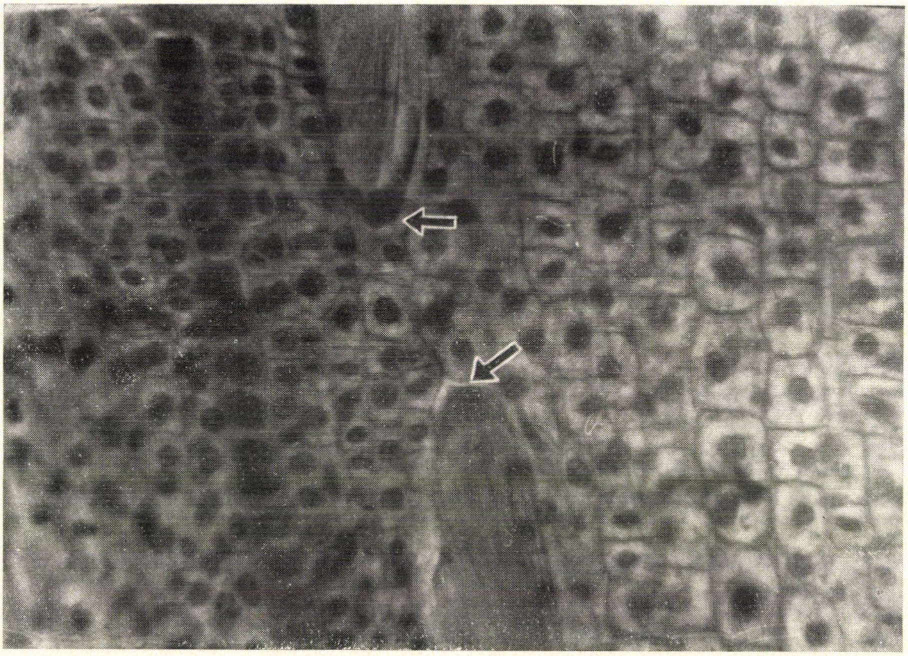

Fig. 8. Detail of a growing idioblast with thickened cell-wall at the apical part (←) LM: 390×

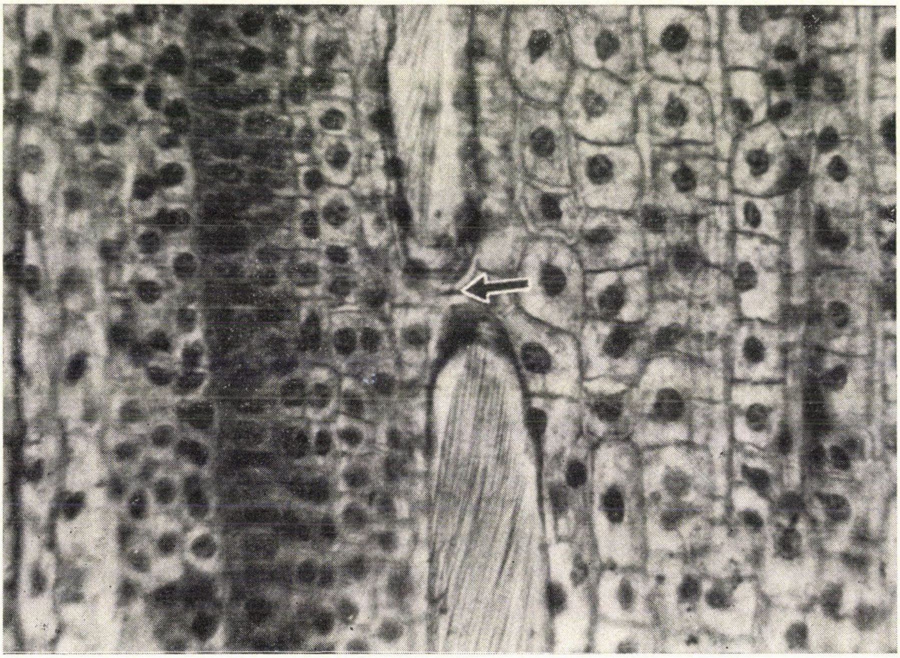

Fig. 9. The adjacent idioblasts touch. LM: 390×

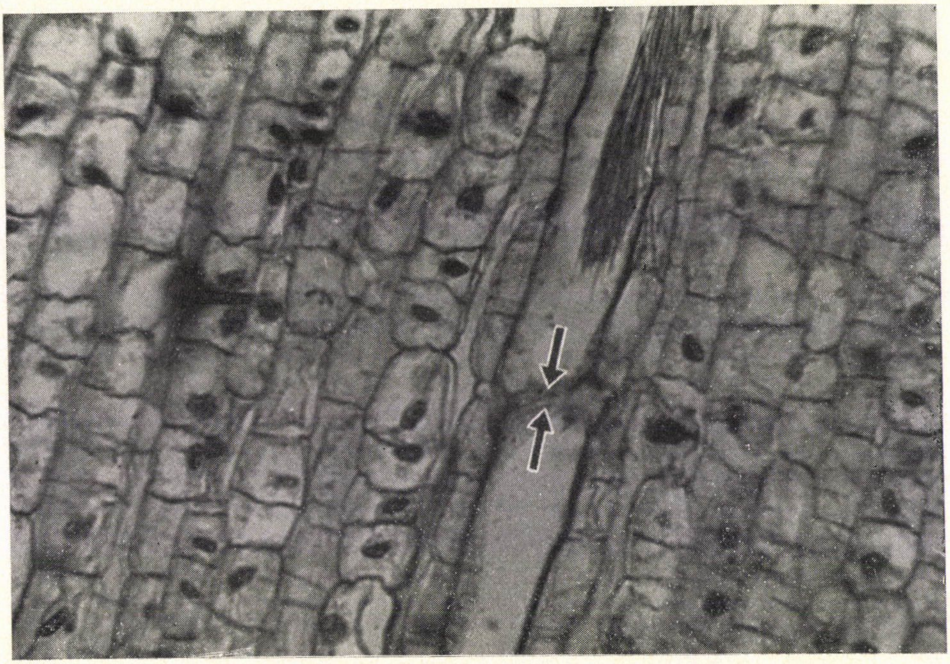

Fig. 10. Adjacent idioblasts with widening transversal walls (←) LM: 390×

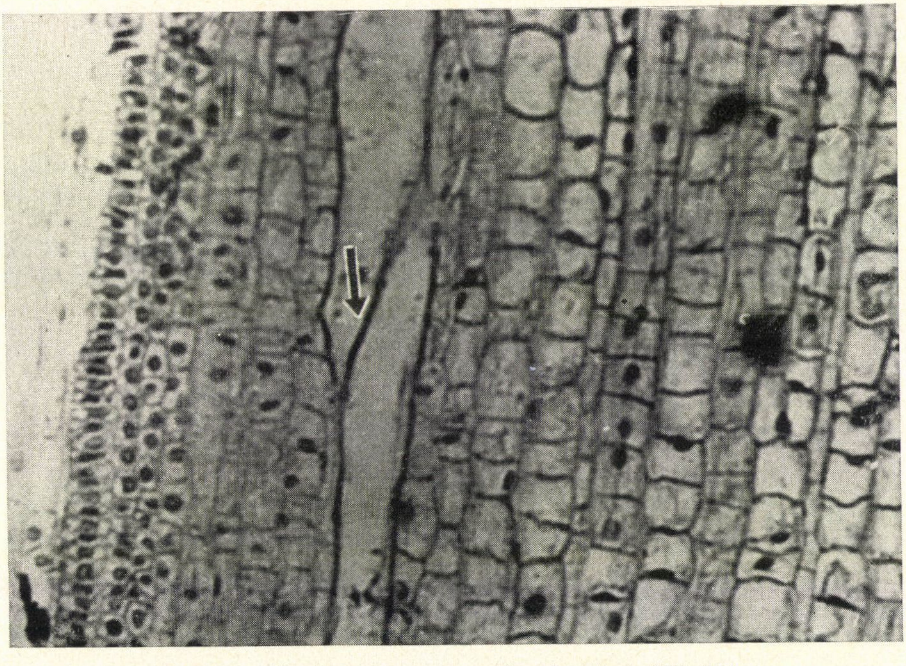

Fig. 11. Idioblasts overgrow each other (←). LM: 320×

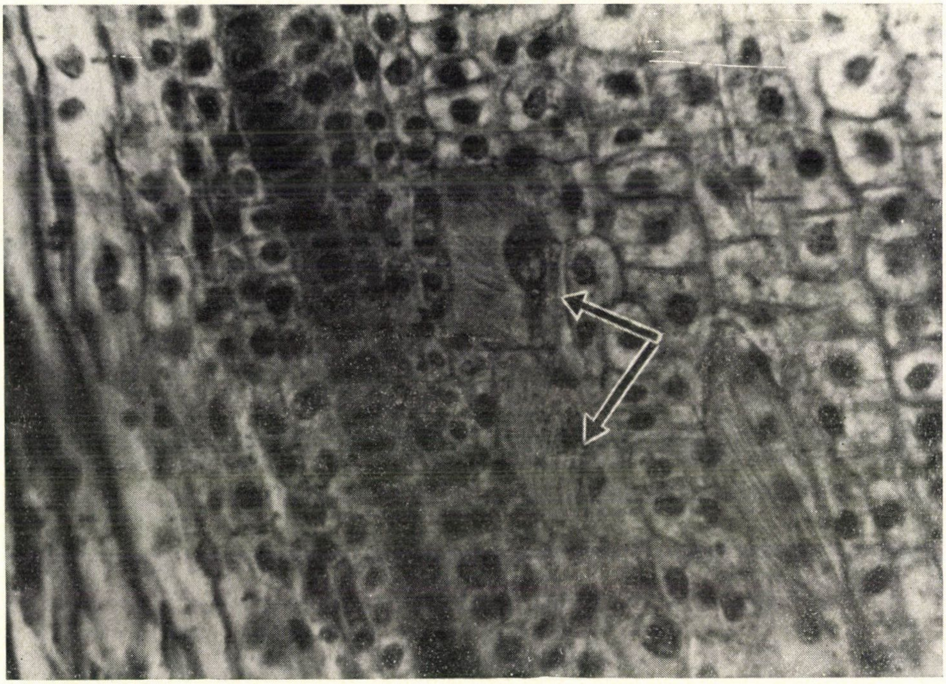

Fig. 12. Twin idioblasts (←) in the periblem (longitudinal section). LM: 390 ×

even attain 3—5 mm. In the course of growing there is a high-degree crystal accumulation until, finally, the cell will be filled with raphides.

For a while the growth of the nucleus keeps abreast with that of the cell and mostly occupies the central part of the cell; later, the nucleus often adheres to one of the side walls.

The cytoplasm can be recognized — especially at the tips — for a long time during the growth of the cell, and together with the nucleus indicates the active functioning of the idioblast. As long as the crystal accumulation lasts, the cytoplasm does not die.

To sum up our investigation we can establish that in the aerial root of *Monstera deliciosa* the crystalliferous idioblasts are produced by unequal cell division. Similar observations were made by KOWALEWITZ (1956) who found young raphide idioblasts in the embryonal tissues of *Epibolium* flower buds.

In most cases multiple-size nuclei occur in the idioblasts which can be the consequence of endomitosis. We also found — though less frequently — two smaller nuclei in one cell close to one another, similarly to the observation of RENNER *et al.* (1952). Our material offered examples of Schötz's twin crystal-idioblasts too (SCHÖTZ *et al.* 1970). This suggests that in the aerial root of *Monstera deliciosa* the raphide idioblasts develop first of all in such a way that after the

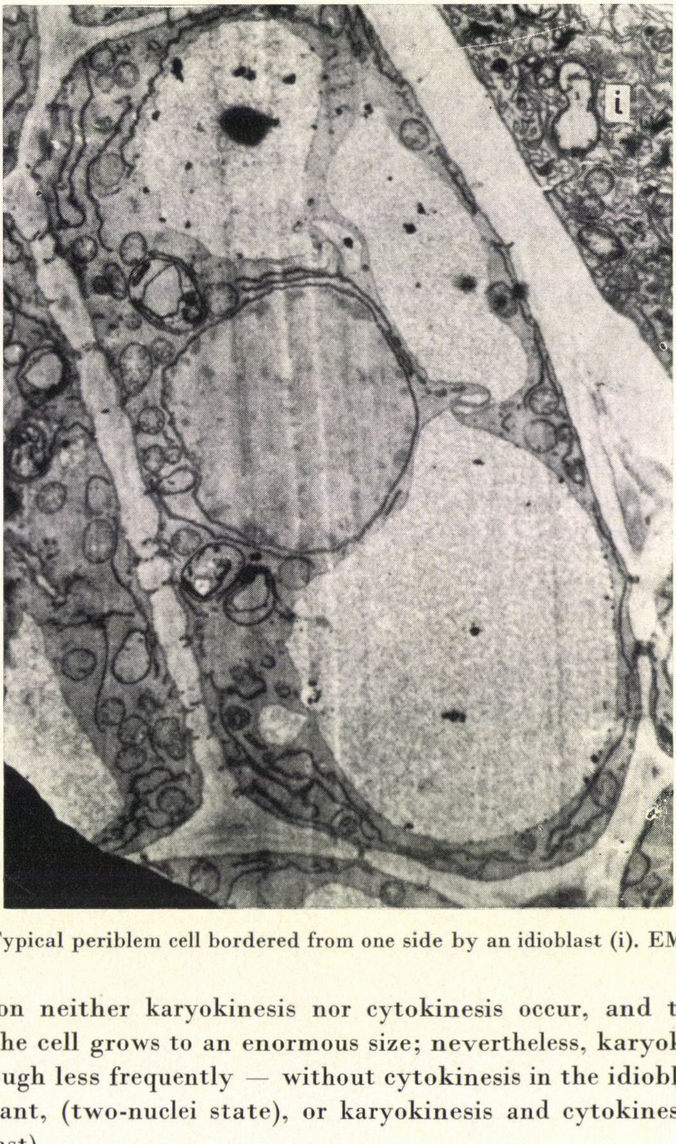

Fig. 13a. Typical periblem cell bordered from one side by an idioblast (i). EM: 5500×

differentiation neither karyokinesis nor cytokinesis occur, and the nucleus along with the cell grows to an enormous size; nevertheless, karyokinesis may occur — though less frequently — without cytokinesis in the idioblasts within the same plant, (two-nuclei state), or karyokinesis and cytokinesis together (twin-idioblast).

Arnott (1962) found a linear arrangement of the crystal-idioblasts in the root tip of *Yucca* but did not give an explanation for this way of development. In our opinion the linear arrangement can be traced back to the determination and differentiation of periblem cells found in certain definite places.

Kowalewitz (1956) supposed an apical growth to take place during the development of the raphide idioblasts on the basis of the cytoplasm remaining thick and the cell-wall thin for a long time at the tips. We, on the contrary, found thick cell-walls at the tips of the idioblasts; and this fact — even if not

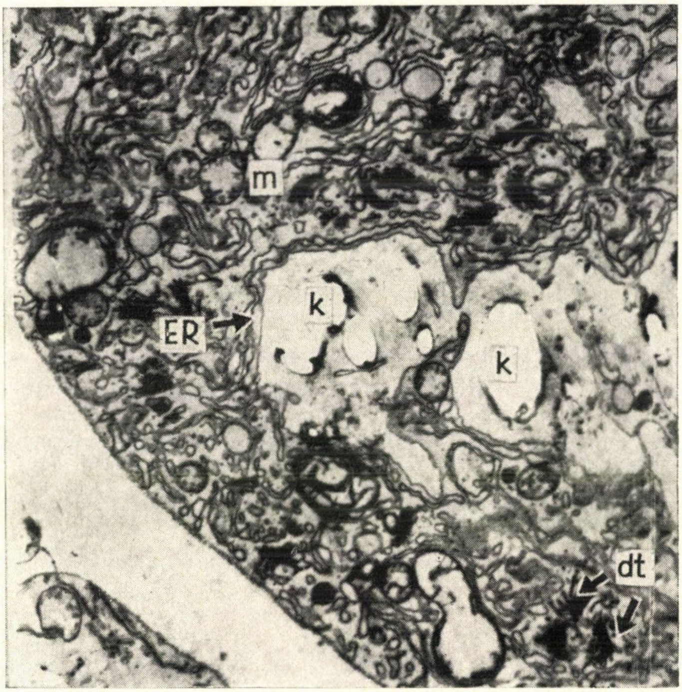

Fig. 13b. Larger part of the idioblast bordering the right upper corner of the periblem cell in Fig. 13a.: place of crystals in the vacuole (k), mitochondrium (m), dictyosome (dt), endoplasmic reticulum (ER). EM: 8800 ×

disproving the apical growth — indicates that the idioblasts advance among the cells by their aid.

Kowalewitz (1956) and others did not bring the temporarily enlarged nucleus of the crystal-idioblast into connection with the secretory nature of the cell. According to Mollenhauer—Larson (1966), the active cytoplasm of the crystal-idioblasts can be explained by the secretory activity of the cell. Our investigations suggest that a joint participation of a large nucleus and active cytoplasma is required for the secretory processes.

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DETERMINATION OF LYSINE AND METHIONINE REQUIREMENTS OF PIGS I. AMINO-ACID SUPPLY OF FATTENING PIGS DETERMINED BY FARM-SCALE AND NITROGEN-BALANCE EXPERIMENTS

By

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Fattening- group experiments were carried out with young Hungarian large white pigs, as well as nitrogen-balance tests with albino rats and pigs fed on fodder mixtures used in the group experiment. The percentage of lysine and methionine varied in the fodder mixtures. In the experiments a close correlation was found ($r = 0.9716$) between the consumed quantities of lysine and the values of nitrogen equilibrium. The N-balance experiments are considered suitable for determining the amino- acid requirements of pigs. Furthermore, in a feed mixture of 10 per cent crude protein content and 72 kg/q starch value an addition of 0,9 per cent lysine and 0.2 per cent methionine was found to be necessary. These values of amino acid requirement were significantly proved by the results of group-fattening experiments and N-balance tests.

Introduction

The main purpose of livestock breeding is to increase the production of meat, milk and eggs in order to make a sufficient amount of animal-origin protein available for human consumption. It is generally known that in a part of the world pork forms a considerable proportion of the protein sources of animal origin, and in Hungary, due to the established consumption habits, pork production plays an especially important role.

In pig feeding — as it is known — proteins, their quality and amino-acid composition are of extraordinary importance. If the proteins of feeds do not contain sufficient quantities of amino acids essential for pigs, the amino-acid balance of the organism will be upset. Proteins of inadequate amino-acid composition, when consumed at a higher rate, decrease the output of the animal.

Owing to the different investigation methods, there are differences between the values determined for the amino-acid requirement of pigs. It is further influenced by the breed of the animals, the composition of the feed rations and amino acids, the concentration of protein, the energy content and the quantity of vitamins and material substances.

According to Rerat—Lougnon (1965), the divergent values of lysine requirement found in the literature for the amino-acid requirement of pigs can be explained by the fact that the amino-acid composition of proteins fed in the different countries is not the same, so their biological value is not identical either, further, that in many places a safety surplus of protein is supplied with the feed ration in order to cover the amino-acid requirement under all circumstances.

RERAT—LOUGNON (1968), MITCHELL *et al.* (1968), WIESEMÜLLER—POPPE (1968), SCHILLER (1969), MÜLLER—ROZMAN (1969), TYPPO *et al.* (1970), furthermore STOCKLAND *et al.* (1971) determined the amino-acid requirement of pigs in different ways. The authors — quite independently from one another — point out that the established values always refer to the same breed (type of utilization) alone.

Taking the above in consideration, our investigations were aimed at giving a more precise answer to the question of the lysine and methionine requirements of young pigs under the special keeping and feeding conditions of Hungarian breeds.

Material and Methods

In our experiments we started by feeding a fodder mixture containing both lysine and methionine at a low percentage. This diet was completed in different ways with synthetic l-lysine and dl-methionine. Our feed mixtures consisted exclusively of fodders of plant origin as well as of mineral substances and vitamins. The composition and nutrient content of the diets are shown in Table 1.

The experiments were set up with the following treatments;

1. Group experiments with young pigs. The experiment was carried out in the traditional fattening unit of the Agárd State Farm with Hungarian large white pigs. The experiment was started with animals of 21 kg average weight in five groups, each containing 25 pigs, and completed when the animals in Group 4 had come close to the 100 kg average weight.

2. N-balance tests with rats and pigs. The 5 diets presented in Table 1 were fed to young male albino rats (Wisthar strain) kept simultaneously in metabolism cages. The question to be answered was: What changes would occur in the N balance of rats as a response to the different amino-acid contents of the diets. The examination was performed in two phases;

in phase a) with animals of 80—100 g weight,

in phase b) with ones of 150—160 g weight, in order to find out what influence the different amino acid supplements exercise on the N balance of younger and older organisms, respectively.

Furthermore, nitrogen-balance tests were performed by feeding the diets to 4 hybrid pigs (of 35—45 kg average weight) per group — produced by the Research Institute of Animal Production (AHIB). The animals were kept in metabolic pens, and the amount of nitrogen consumed by them with the feed, and excreted with the faeces and urine was measured every day over a six-day experiment period following six days of pre-feeding — a way usual in the material-balance experiments.

Results

1. Table 1 shows that it was only in the lysine and methionine content that differences were found between the compositions of the five feed mixtures. The crude protein content of feeds did not even reach 10 per cent. This quantity is not enough for young pigs; it decreases the rate of growth, and due to the inadequate ratio of protein to energy (72 kg/q starch value), the animals put on

Table 1

Composition and nutrient content of experimental diets for rats and pigs

Designation		Groups				
		1.	2.	3.	4.	5.
Maize	%	56.5	56.2	55.9	55.4	56.0
Barley	%	40.0	40.0	40.0	40.0	40.0
Vitamin premix	%	1.0	1.0	1.0	1.0	1.0
Mineral premix	%	0.5	0.5	0.5	0.5	0.5
Feeder salt	%	0.4	0.4	0.4	0.4	0.4
Feeder lime	%	1.6	1.6	1.6	1.6	1.6
L-lysine	%	—	0.3	0.6	0.6	—
Dl-methionine	%	—	—	—	0.5	0.5
Total	%	100.0	100.0	100.0	100.0	100.0
Dry matter	%	91.18	92.20	91.10	92.25	90.20
Crude protein	%	9.81	9.69	9.69	9.81	9.81
Lysine	%	0.318	0.617	0.916	0.915	0.318
Methionine	%	0.175	0.175	0.174	0.673	0.673
Cystine	%	0.184	0.183	0.183	0.182	0.183
Starch value	kg/q	72.21	71.97	71.74	71.34	71.82

weight (fat deposition). On the other hand, it was only in that way that we could achieve various mixtures with synthetic l-lysine and dl-methionine, by supplementing the feed lysine and methionine concentrations in them.

Table 2 contains the experimental data obtained during the fattening.

There are great differences both in the average weight gain and in the feed conversion between the results of the five groups. These differences are due to the following:

The output of animals was increased by both the 0.3 and the 0.6 per cent (Group 2 and 3) 1-lysine supplement. The daily weight gain of pigs given a 0.3 per cent lysine supplement increased by 25 per cent, while the amount of feed used for 1 kg weight gain decreased by 17 per cent compared to the control. The higher rate (0.6 per cent) of lysine supplementation gave even more favourable results, increasing the weight gain by 32 per cent and decreasing the amount of feed used for 1 kg weight gain by 20 per cent.

The results in Group 4 were roughly the same as those in Group 3. A 0.5-per cent methionine supplement given simultaneously with the 0.6-per cent lysine supplement neither improved nor impaired the output of animals. From this we have drawn the conclusion that a methionine supplement is not always needed.

Table 2

Results of experiments performed with fattening pigs

Parameter		Groups				
		1.	2.	3.	4.	5.
Average initial weight	kg	21.60	21.20	21.20	21.40	21.20
Average final weight	kg	80.68	91.67	97.80	99.79	79.39
	%	100	114	121	124	98
Total weight gain	kg	59.08	70.47	76.60	78.39	58.19
Daily weight gain	g	453	568	598	605	458
	%	100	125	132	133	101
Feed used per animal	kg	248.09	246.20	257.20	266.20	235.44
	%	100	99	104	107	95
Feed used for 1 kg weight gain	kg	4.20	3.69	3.36	3.40	4.05
	%	100	83	80	81	96

The feed mixture of Group 5 contained 0.67 per cent methionine and 0.32 per cent lysine. This diet was consumed by the animals for 95 days. By that time their health conditions deteriorated, their feed conversion and weight gain fell considerably. Having attained good results with the feed mixture of Group 4, we continued to feed the animals in Group 5 on the diet of Group 4. Characteristically of the difference between the two groups, after 95 days of fattening the average weight of the animals was 80 kg in Group 4 and 65 kg in Group 5. At the end of the experiment the weight of the animals in Group 5 corresponds to the weight of those in Group 1 (control), but the results are poor in both groups, for which the inadequate lysine percentage may be responsible.

The trend of the weight gain in the group experiment of young pigs is shown in Fig. 1.

2. The results of nitrogen-balance tests carried out with albino rats are shown in Table 3.

With rats the best results were obtained by feeding a 0.3 per cent lysine supplement (feed mixture No. 2). The results obtained in the N-balance tests of rats are presented in Fig. 2.

The results of N-balance tests carried out with 4 pigs per treatment given the above listed feed mixtures are shown in Table 4.

In comparison with Group 1 (control) the nitrogen retention of animals consuming Diets No. 2, 3 and 4 was significantly better ($P < 0.1\%$). The N-balance values differed from the results of the fattening experiment inasmuch as it was with the results of Group 2 that the N-retention values of Group 4 were nearly identical.

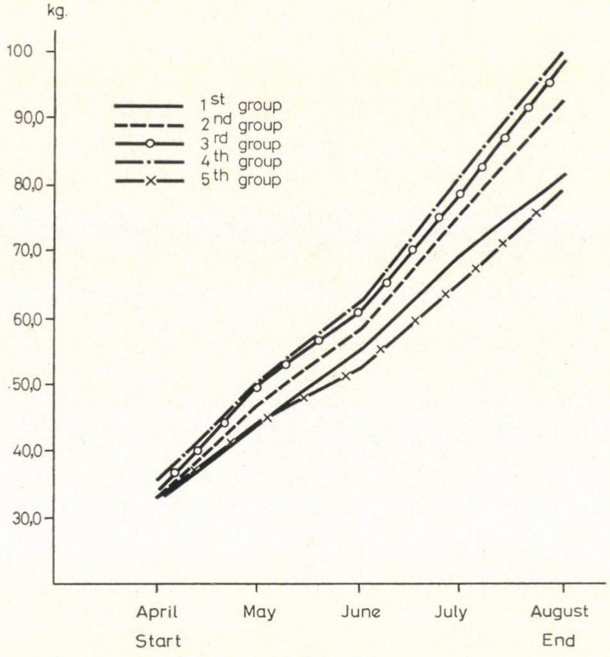


Fig. 1. Monthly gain of pigs during the experimental period

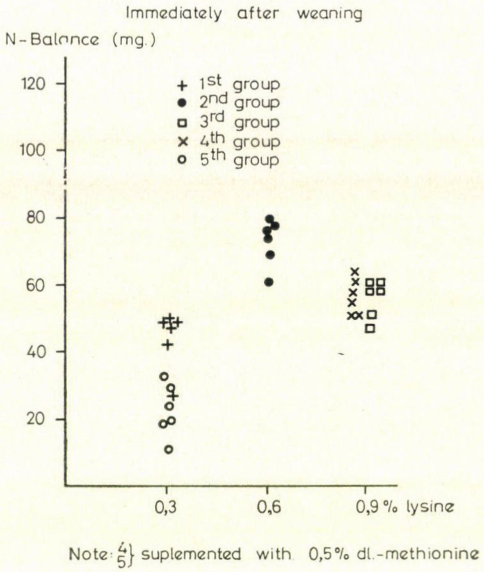
According to the fattening experiment the results attained with the 10% crude protein content diet in which the percentage of lysine was 0.92 and that of methionine 0.67 are just as good as those obtained with a methionine content of 0.17 per cent beside the 0.92 per cent lysine.

Diet No. 5 was not examined in N-turnover tests with pigs, since bad results were obtained during the fattening and a rather negative N-balance in the experiment performed with rats.

Table 3

Results of N-balance tests performed with rats

Group	Daily nitrogen balance			
	initial		final	
	mg	%	mg	%
1.	45±2.65	100	71±4.7	100
2.	73±2.77	162	116±3.8	163
3.	57±2.47	127	83±5.3	117
4.	57±2.03	127	91±3.1	128
5.	23±3.20	51	41±2.6	58



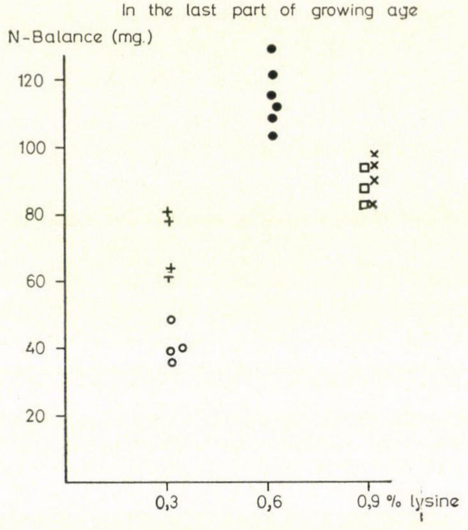


Fig. 2. N balance of growing rats in function of lysine intake

In the nitrogen-balance tests the best results were attained with Group 3. The diet of this group was only supplemented by lysine. According to the results, in the present case the methionine supplement decreased the rate of feed conversion in the pigs. The results of nitrogen-retention experiments with pigs are shown in Fig. 3.

The nitrogen-turnover tests carried out with rats and pigs confirm the results of the fattening experiments. A properly performed N turnover test

Table 4

Results of N balance tests performed with pigs

Animal	Daily nitrogen balance in g			
	Diet 1.	Diet 2.	Diet 3.	Diet 4.
I.	1.80±1.82	6.99±2.61	20.37±5.44	5.51±0.80
II.	2.07±1.99	9.55±1.52	19.35±3.86	7.01±1.37
III.	2.00±1.35	8.27±2.10	19.78±0.94	7.41±0.68
IV.	1.19±1.08	11.75±1.56	22.78±1.57	7.10±1.17
$\bar{x}$	1.77±0.40	9.14±2.03	20.72±1.40	6.76±0.85

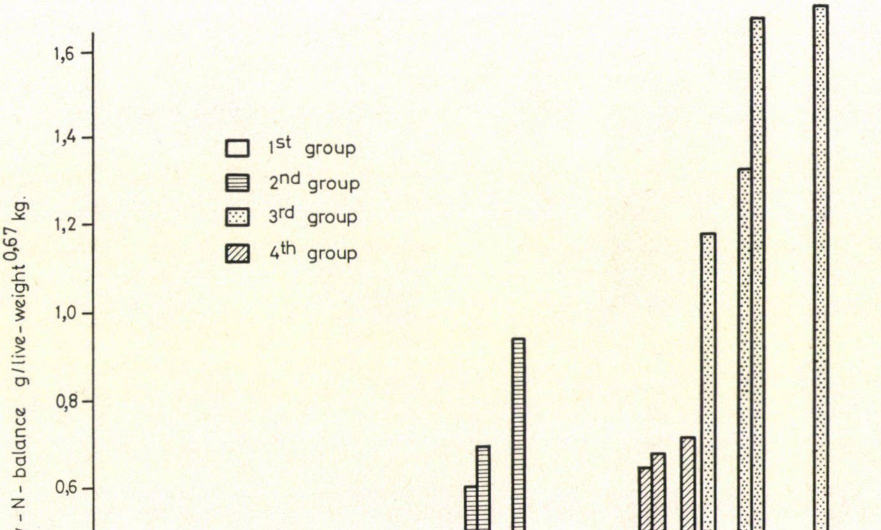


Fig. 3. The effect of lysine and methionine intake on N retention of growing pigs

is suitable for determining the amino-acid requirement of pigs. Namely, a close correlation (r = 0.9716) between the lysine content of the feed and the N retention of the animals is clearly seen from the results.

Hence, as a final conclusion drawn from the results of our investigations, young large white Hungarian pigs require 0.9 per cent lysine and 0.2 per cent methionine added to their feed mixture of 10 per cent crude protein content and 72 kg/q starch value.

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STIMULATIVE EFFECT ON PROTEIN SYNTHESIS OF MAGNESIUM APPLIED BY FOLIAGE SPRAY

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The incorporation in protein of amino-nitrogen applied through foliage spray was considerably stimulated by simultaneously added magnesium ions. The accumulation of nitrogen in protein was especially increased by the magnesium in the first three hours following the treatment. The indirect effect of magnesium in stimulating the protein synthesis can supposedly be traced back to two factors. One of the effects of magnesium supplementation is felt in the maintenance of the polyribosomal structures, which is a limiting condition of peptide synthesis, while in the aminating reactions magnesium, as an activator, reduces the demand for a change of free energy in transformations, in a bioenergetic respect. For the theoretical interpretation of the stimulative effect of the magnesium ion on protein synthesis the above two action mechanisms provide a satisfactory explanation.

Introduction

The relevant data of literature suggest that the mechanism of nutrient uptake from solutions by the leaves agrees in essentials with nutrient uptake by the roots (Matzkov 1962, Wittwer—Teubner 1959).

Kerpely (1913), the Hungarian pioneer of leaf nutrition has been followed by many. Experiments were carried on first of all with nitrogen application to wheat (Ferencz 1954, Kuthy *et al.* 1951), vine (Pecznik—Majer-Kiss 1958), silo maize (Pecznik—Dorogi 1963). Pecznik *et al.* (1958, 1963) pointed out that as a response to nitrogen (urea) applied through foliage spray, not only the quantity of yield but also the protein content increased. Starting from this fact, as well as on the basis of literary data and or ouwn experiences (as to magnesium stimulating the protein synthesis), we supposed that an urea nutrition combined with magnesium would increase the protein level of leaves even more. On this basis we studied the joint effect of nitrogen and magnesium on protein increase in millet and pea test plants. Our reason for choosing plants from two highly dif- ferent families was that Domonovics—Zselenov (cit. Matzkov 1962) — when studying the uptake of potassium and magnesium by the leaf — had found the various plants to give different responses to foliar spray.

Material and Method

In our experiment a commercial pea variety (Express) and millet were used as test plants. The treatments were applied to both plants at the same phenophase, immediately before flowering and the appearance of panicles, respectively. The experiment was carried out at the Agrochemical Experimental Station of the Borsod Chemical Works, with four replications.

Treatments;

Control (initial state)
N: 1.3g urea + 260 ml water/m^2
N + Mg: 1.3 g urea + 0.13 g $MgSO_4$ + 260 ml water/m^2

Spraying was performed with a finely atomizing "Nebulo" sprayer, in cloudy weather. Samples were taken 3 and 72 hours after the treatment, respectively. The reason why the first sample was taken 3 hours after the treatment was that according to the experimental results of FERENCZ (1963) obtained with ^{14}C, the first effect was felt 20 minutes after the spraying in a temporary reduction of photosynthesis. During this transitional state the protein synthesis increased as measured by the incorporation of ^{14}C. In a few hours the normal rate of photosynthesis was restored, and later even increased. It was in that very state of reduced photosynthesis that we wanted to study the intensity of protein synthesis, that is, why we chose the third hour following the treatment as the time of sample taking. And the same state was considered the most suitable for proving the enzyme-activating effect of magnesium as well. When taking samples, the plants were cut off at a height of 5 cm above ground level. The spray not yet absorbed by the leaves was washed off the plants, then the whole plant was dried out in an exsiccator at 105 °C. After a rough crushing the sample was reduced by a quarter, then ground in an electric mill. For the examination of the protein nitrogen and the total nitrogen content, and by Kjeldahl's sulphuric acid destruction of nitrogen compounds precipitable with copper, the ammonium nitrogen was determined (GYŐRI—IHÁSZ 1968). The nitrogen contents thus obtained were converted related to the crude (total nitrogen), and true protein (protein nitrogen), respectively.

Results

The results of investigating the increase of protein as a response to amino nitrogen and amino nitrogen + magnesium applied as a foliage spray are shown in Table 1. The results of the experiment significantly (S. $D._{5\%}$) prove

Table 1

Effects of N and N+Mg nutrition applied as foliage spray on the protein contents of pea and millet plants

Plant treatment	Protein content as a percentage to dry matter							
	At the start		3 hours later			72 hours later		
	crude	true	crude	true	stimulation %	crude	true	stimuation %
	protein		protein			protein		
Pea control	22.2	16.1	—	—	—	—	—	—
N	—	—	23.5	16.6	3.1	25.4	20.9	30.0
N + Mg	—	—	24.5	18.5	15.0	26.2	22.1	37.5
Millet control	19.6	16.7	—	—	—	—	—	—
N	—	—	22.0	18.0	7.7	22.8	18.3	9.5
N + Mg	—	—	23.5	19.1	14.4	23.2	19.0	13.7
$S.D._{5\%} = 0.4$								

the stimulative effect of magnesium on the protein synthesis and protein levels. It was manifest first of all in the stimulation of the true protein content in pea which at the beginning was more than three times the effect of nitrogen. After 72 hours the difference essentially decreased, the effect of the nitrogen treatment was close (only 27 per cent lower) to that of N + Mg applied jointly. This "approximating" effect can be explained by the law of equilibrium, that is, by the concentration of incorporable amino nitrogen decreasing with time. It was partly by this that we proved the stimulative effect of magnesium on the protein level, and its role as an enzyme activator. This explanation is justified by the effect of magnesium playing a role in the ribosomal structural relations of protein synthesis.

Interpretation of stimulation caused by magnesium. The effect of magnesium as an activator inducing the stimulation of protein synthesis can — in our opinion — be explained by its influence on the ribosomes and the bioenergetic role of ATP synthesis. PETERMANN (1964) thought it probable that protein synthesis was the only task of the ribosomes discovered by PALADE (1956). McCHARTY (1962) proved by concrete measurements that the extent of protein synthesis was in proportion to the number of ribosomes. Magnesium has an important part in maintaining the structure of ribosomes. The structural wholeness of the ribosome requires a magnesium concentration of 10^{-3} mol. If the concentration of magnesium decreases to 10^{-4} mol, the polyribosome will be fragmented at a ratio of 2 : 1 into subunits. So the ribosome of 70 S sedimentation will decompose into subunits of 50 S and 30 S, at the above ratio. If the magnesium concentration is increased to 10^{-2} mol, the two ribosome fractions with different subunits will unify, dimerize (DE ROBERTIS *et al.* 1970).

According to Wattson, neither the subunits nor the dimers are able to condense the amino acids into peptids. TRAUB—NOMURA (1969) think the formation of subunits and dimers to be reversible and dependent on the concentration of magnesium.

In the course of an electron-microscope study, PALADE (1956) pointed out the ribosomes to be interlinked in the polyribosome as the pearls of a necklace, but only in the case of the critical magnesium concentration of 10^{-3} mol. According to earlier data the protein synthesis takes place in the polyribosome chain, with the aid of measuring the incorporation of isotope-labelled amino acids. This is one of the evidences of the relationship between structure and function. After this it is necessary to have a look at the role of magnesium in developing the structure of the ribosome. As known from the investigations of WEBSTER—WHITMAN (1961) the ribosome is built of protein and RNA at a rate of 55 : 45 in clover, 60 : 40 in rat liver and 50 : 50 in the reticulocyte of rabbit. GOLDBERG (1966) pointed out that the proteins were linked with the RNA by magnesium ions. He further proved with *E. coli*, pea and rat liver ribosome in vitro that the bound magnesium was a function of the concentration of free magnesium ions in the medium. The quantity of magnesium bound by the 70 S ribosome of *E. coli* is three times more in the case of a 10^{-2} mol magnesium concentration of the medium than when the concentration is only 10^{-4} mol. In the latter case dissociated (30 S and 50 S subunits) ribosome were present in the medium. Goldberg found, at the same time, that at concentrations of 10^{-4}—10^{-2} mol a basic ribosome fixed about 1600 magnesium ions, at a ratio of 0.5 Mg : P. On this basis it can be supposed that the phosphate of a complete ribosome is linked with magnesium. In certain cases more than one magnesium atom may be attached to the RNA, which is in connection with the high ion intensity due to the phosphate group of RNA. According to HURWITZ—ROSANO (1967) magnesium occurs on the ribosome in two types of bond. One of them can be replaced through dialysis (to about one third) while the other cannot. Magnesium fixed with a stronger bond belongs to the laminar RNA. According to investigations made by the authors the stability of the ribosome originating from the embryo of the rice plant depends not only on the magnesium concentration, but also on the temperature. At a temperature of 0 °C dissociation into subunits only occurred with a magnesium concentration of $1 \cdot 10^{-4}$, mol., while at 28 °C it came about already at $5 \cdot 10^{-4}$ mol.

Here we present an aminating reaction, as the scheme of glutamin synthesis:

$$\text{Glutamate}^{2-} + \text{ATP}^{4-} + \text{NH}_4 \longrightarrow \text{Glutamine}^{-} + \text{ADP}^{3-} + \\ + \text{HPO}_4^{2-} + \text{H}^{+}$$

This reaction is catalysed by the glutamine-synthetaze enzyme which, in turn, is activated by magnesium.

On the basis of microcalorimetric studies, Robinson pointed out that under identical conditions the free energy change in a medium containing magnesium was —0.7 kcal/mol, while in the absence of magnesium —9.34 kcal/mol. Since the aminating processes are important reactions of the protein synthesis, it is easy to understand that magnesium plays a significant role in the energy turnover in spite of the fact that the acid amines can only be regarded as amino reserves.

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SOME OBSERVATIONS ON THE SPERMOSIN FRACTIONS

EXCITATION AND FLUORESCENCE SPECTRA, AND AMINO ACID COMPOSITION OF SPERMOSIN AND ACTOSPERMOSIN FRACTIONS

By

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In our experiments we deal with the fraction corresponding to actospermosin and with the chromatographic fractions of spermosin. In the course of fractioning we followed the disproportioning of the tail part of the spermatozoa which we now present in light- and electron-microscopic photos. We studied the excitation and fluorescence spectra as well as amino acid composition of chromatographic actospermosin and spermosin fractions. The results show that the excitation spectrum of actospermosin is restricted to a rather narrow band, but its fluorescence spectrum has a broad surface even in the vicinity of the peak which shows that it is not a uniform component. Before chromatography the excitation and fluorescence spectra of the spermosin fraction suggest heterogeneity which is proved by the six fractions of the separation. Fractions III, IV and V display a considerable ATP-ase activity, while the activity of fraction II has not been measured yet. In spite of the separation, the excitation and fluorescence spectra of the individual fractions still indicate heterogeneity. Methylated lysine occurs in the composition of the actospermosin and fractions IV and V of the spermosin: actospermosin shows approximately 1 M%, fraction IV 0.76 and fraction V 1.22% methylated lysin. The comparative basic amino-acid standard chromatograms show that the methylated amino acid of the actospermosin and spermosin fractions is ε-N-trimethyl lysin.

Introduction

The purification of bull spermosin was first attempted by Engelhardt—Burnasheva (1957), Burnasheva (1958) whose preparations were not, however, sufficiently pure, owing to methodical difficulties. In an earlier paper (Fazekas—Veres 1971) we described that spermosin — a fraction corresponding to isomyosin — could be produced from the tail fraction of bull spermatozoa in chromatographic purity. With the described method the crude spermosin eluates from the DEAE column in fraction IV, displays ATP-ase activity and a tendency to complex formation.

The present paper gives an account of the excitation and fluorescence spectra of crude and chromatographically purified spermosin fractions with the view of comparing them with the excitation and fluorescence spectra of myosin fractions obtained earlier in a similar way (Fazekas *et al.* 1971).

Besides, we also determined the qualitative amino-acid composition of actospermosin and spermosin by amino-acid analysis, and attempted to prove the occurrence and assess the quantity of methylated amino acids by column and layer chromatography.

Material and Method

Ejaculate freshly obtained from 5—8 bulls was cooled to 0 °C in 0.6 × 12 cm glass tubes put in ice, diluted with 0.1 M NaCl to a fourfold volume, then the cellular elements collected by centrifuging. The residue was suspended with NaCl solution of fivefold volume, and centrifuged to remove the soluble parts left behind. The residue containing the washed spermatozoa was suspended in 0.15 M KCl containing 20 mM TRIS-HCl to a tenfold volume (pH 7.2), separated into head and tail fractions with ultrasonication (7 kilocycle, 10 minutes), and the head fractions were collected after 3 minutes of centrifuging at 300 g. A microscope examination of the greyish residue revealed occasional tail fractions. Therefore, this fraction was again suspended, and — as before — differentiated in a centrifuge in order to pass over the remaining tail fractions to the supernatant. From the joined supernatant fraction the tail parts were removed by centrifuging (for 20 minutes at 5000 g). The fractions thus collected were used for the isolation of spermosin — with some modification as described in an earlier publication (Fazekas—Veres 1971). The tail fraction was suspended in 5 ml 0.15 M KCl and repeatedly disintegrated with ultrasonic treatments (10 minutes, 7 kilocycle) to facilitate the isolation of spermosin in the next step. The volume of the suspension was diluted with appropriate solutions to 20 times the volume of the solid material so that it contained 0.5 M KCl, 0.11 M phosphate buffer (pH 6.3) and 2 mM 2-mercaptoethanol. During the extraction the suspension was put in ice and now and then stirred, then the insoluble residue was removed after 20 minutes by centrifuging (for 20 minutes at 5000g). From the supernatant the spermosin was precipitated by dilution with distilled water at a ratio of 11 : 1, and collected by centrifuging for 20 minutes (5000g). The precipitate was redissolved in 0.5 M KCl of 15 unit volume — first by stirring it slowly in a low volume suspension —, then the fraction analogous to actomyosin was diluted with distilled water to 0.26 M KCl and thus precipitated, then collected by a 20 minute centrifuging at 5000g. The spermosin left behind in the supernatant was again precipitated through dilution to a concentration of 0.03 M KCl, then collecting by means of a centrifuge, as before. Finally, the crude spermosin was redissolved in 0.5 M KCl containing 2 mM 2-mercaptoethanol, corresponding to a concentration of 5—10 mg/ml.

The spermosin fraction, after sampling, was equilibrated, by being dialysed against 0.02 M pyrophosphate, and purified chromatographically, as described in the previous publication. The results and eluates are presented in Fig. 6.

From the chromatographic fractions we equilibrated the peak tubes and the non-chromatographied samples as well as the fraction called actospermosin by dialysing it against 0.5 M KCl. We determined the UV-absorption values of the fractions at 280 nm, and from an aliquot quantity measured the N content by Kjeldahl's method to be able to calculate their protein content. The N contents were taken uniformly for 16 per cent.

The fluorescence and excitation spectra were measured with a HITACHI MPF-2 A spectrofluorometer at the Institute of Applied Chemistry of the Budapest Technical University. According to a conventional agreement, when measuring the fluorescence spectrum, the optical system was adjusted at right angles to the pathway of the excitation ray of light. Measuring was performed into quartz cuvettes of 1 cm ray length. To remove disperse light, a cut-off filter was applied at 290 nm. A 150 W xenon lamp was used as a light source. When preparing the excitation spectra, we measured the samples at 340 nm, the wavelength of fluorescence maximum. Intensity was marked on the ordinate in relative work units.

The protein samples were hydrolysed with 6 n hydrochloric acid of 1 : 100 ratio in sealed tubes put in a vacuum at 110° for 24 hours. The hydrolysates were dried in a rotary evaporator and used for quantitative analysis.

The amino acid analysis was carried out with a JEOL (Japan) JLC-5AH-type apparatus. We placed the hydrolysates on LC-R-1 type resin made in Japan and employed the method of Selly (1969) in order to be able to detect the methylated amino acids too; chromatography was carried out with 0.35 M citrate buffer (pH 6.58) at 25 °C, with a through-flow speed of 100 ml/hour (= 1.54 ml/min) of the buffer. ε-N-methyl lysin and arginin derivatives were

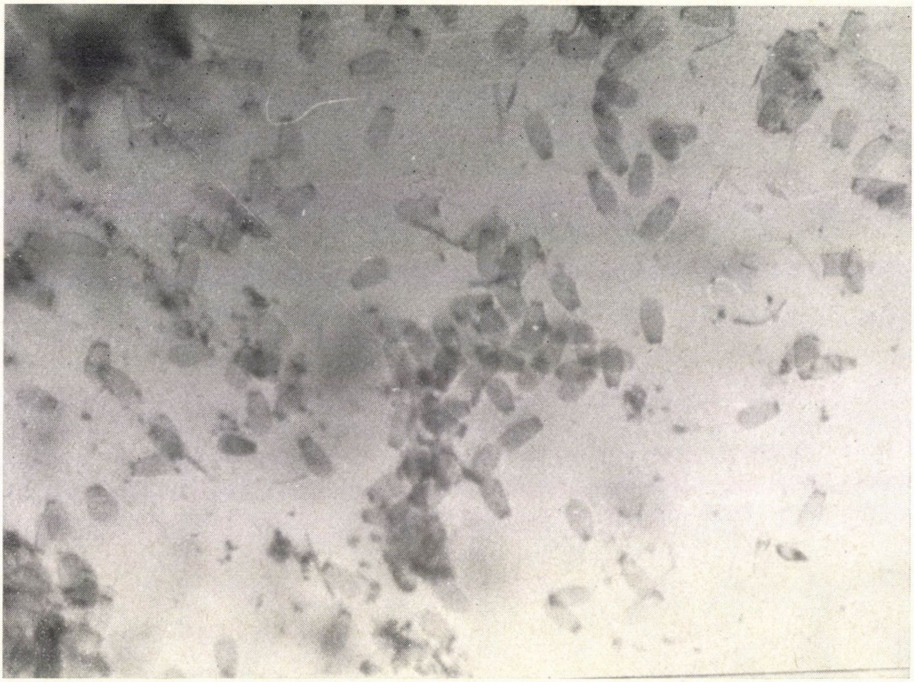

Fig. 1. Head fraction of bull spermium after ultrasonication and centrifugation

earlier produced by Puskás—Tyihák (1969) with total synthesis. With the authentic N-methyl amino-acids and basic amino-acids we performed standard chromatographic separation to identify the unknown amino acids.

The electron-microscopic photos were made with a Phillips EM 200 apparatus after dialysis performed against 1 M ammonium acetate and negative contrast staining with 1 per cent uranyl acetate.

Results

Fig. 1 shows the fraction of spermium heads separated by centrifugation with some tail parts left behind, which justifies us in having repeatedly suspended it and separated it by centrifugation.

To compare the head and tail parts of the spermatozoa, Fig. 2 shows the picture of a spermatozoa made by interference-microscope technique. The head part is about 7—8 micron long, and their mass is seen on the light-microscope photo of Fig. 1. The tail part is 45—55 micron long, and the diameters of the base, middle and bare end part are 0.75—0.85, 0.5 and 0.25 micron, respectively. The most conspicuous feature of the tail part is that the cell membrane and the darkish coat become thin here and there, thus dividing the tail part into 2.5—5.5 micron long members. Therefore the ultrasonic treatment cannot result in intact tail fractions, only smaller or larger parts can

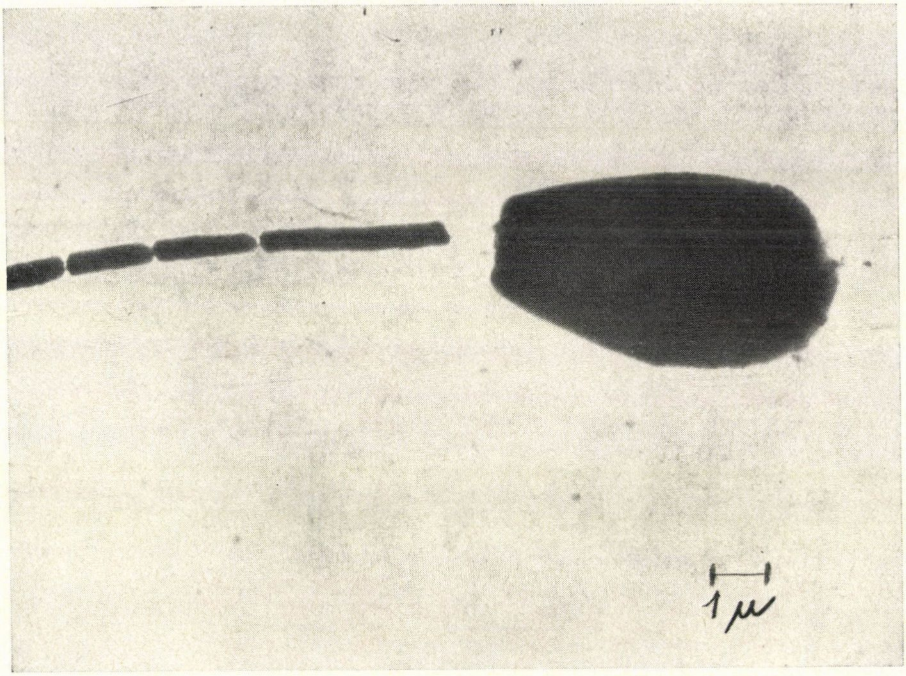

Fig. 2. Interference contrast microscopy of bull spermatozoa

be obtained with it. Fig. 3a shows a piece where we can even see that disproportioning occurs at the places where the tail part is thin. Further, we can see that the tail is of fibrillar structure. The fibrillar system is imbedded in the coat and in some fine basic substance — the matrix. It is known that in the structure of the spermatozoa tail a pattern consisting of 9 outer and 9 inner periferial filaments arranged in two circles, and of two central filaments is realized (BAHR—ZEITLER 1964, BERNSTEIN—TEICHMAN 1972). The figure shows 1 filament of each of the outer and inner circle. Filaments belonging to the outer circle are thicker and more compact. The filaments of the inner circle are thinner. The thicker filaments are seen to be divided into even smaller units by a contrast material stained more dark. On the thinner fibres — where the contrast material has dissolved — it is quite clearly seen that the fibril is divided into sausage-like subunits (subfiber). When examined more closely, these subfilamentary subunits are seen to be divided longitudinally by a dark line, that is, the subfibers of the inner circle are doublets. When taking the measurements of the subfibres into account, we may assume that maximum 6—8 of them can be contained in an inner piece of fiber. Fig. 3b shows magnified subfilamentary subunits.

The 108,000 ×magnification of Fig. 4 shows the doublets to open and

Fig. 3a. Electron-microscopic photograph of the negatively stained fragment of bull spermatozoa's tail fraction. Magnification ×20,000

Fig. 3b. Subfibers of the bull spermatozoa's tail filaments. Magnification ×56,000

reveal a structure consisting of protein molecules arranged regularly like a string of pearls.

On the basis of what have been told above we are justified in having introduced the method of repeated disintegration (ultrasonic treatment). In the course of the subsequent extraction the 0.5 M KCl releases further subunits from the filamentary subunits, and their mass forms the crude spermosin fraction seen in Fig. 5. The subunits seen in the pictures — when compared to those of the subfiber seen in Fig. 3a — appear to contain about four of these elementary units.

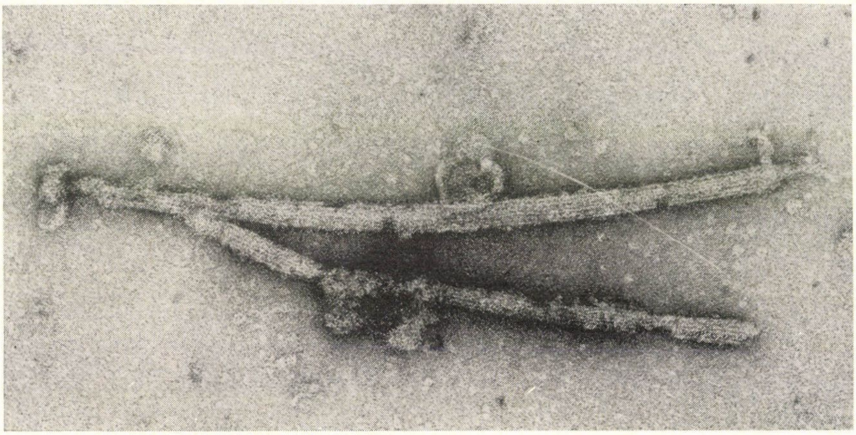

Fig. 4. The doublets of subfiber from inner filament. Magnification ×108,000

Since the salts were removed through dialysis against 1 M ammonium acetate, in some places of the picture salt precipitation from the buffer or the dialysing solution can be seen.

Fig. 6 shows the chromatographic fractions of spermosin separated on a DEAE-cellulose column. The chromatogram differs from the corresponding chromatogram published earlier, inasmuch as we now succeeded in obtaining measurable quantities of fraction III too, so we can present its spectra as well.

Figs 7 and 8 show that the chromatographed spermosin fractions in the peak tubes, having been stored for some 24—36 hours, possess spontaneous aggregation tendencies, and artificial filaments are produced. When examining these artificial filaments more closely, we can see that they do not show a regular pattern, but are spontaneous aggregates.

Fluorescence spectra. Fig. 9 presents the excitation and fluorescence spectra of acrospermosin, Fig. 10 those of spermosin, the latter before the chromatographic separation. In Figs 11 and 12 the spectra of fractions III and IV obtained from the DEAE-cellulose column are seen.

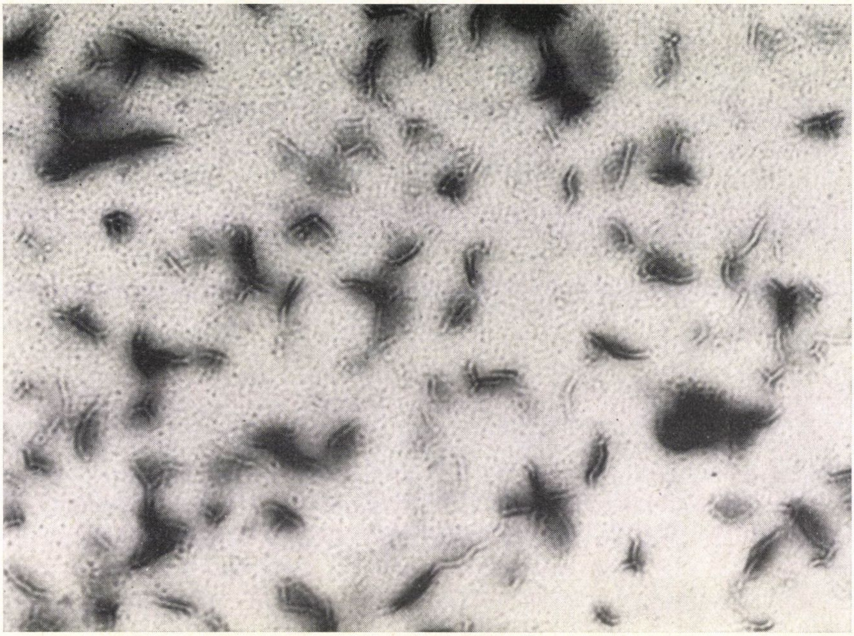

Fig. 5. Protofibrils of bull-tail filaments gained as rough spermosin fraction. Magnification ×16,000

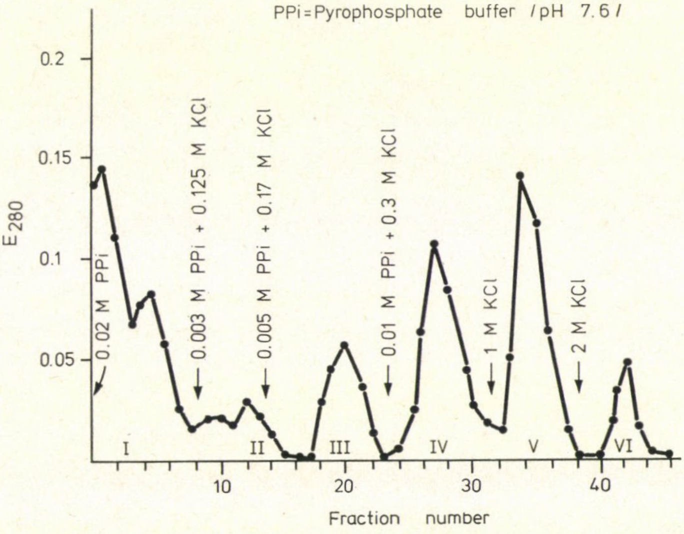

Fig. 6. Chromatography of the bull spermosin on DEAE-cellulose column with step-by-step elution method

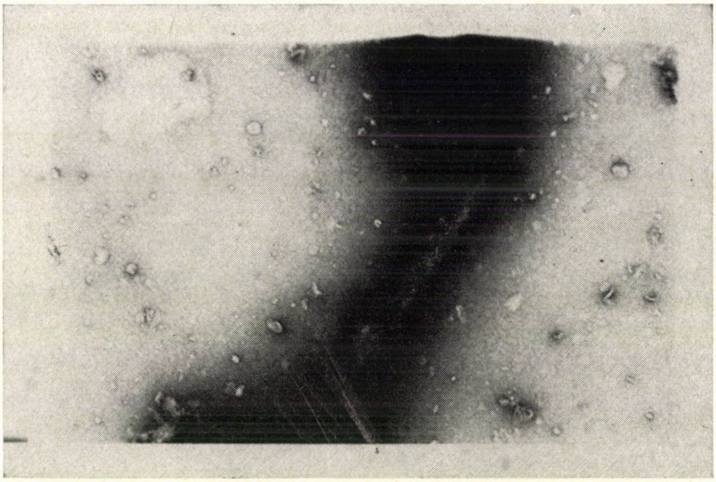

Fig. 7. Aggregated filament from chromatographied spermosin IV fraction. Magnification ×27,000

Fig. 8. Aggregated filament from chromatographied spermosin V fraction. Magnification ×16,000

When comparing the excitation and fluorescence spectra of the individual figures, we can see that the excitation spectrum of the fraction corresponding to "actospermosin" is restricted to a rather narrow band, its maximum being 290 nm, but its fluorescence spectrum is broad even in the vicinity of the peak, and in its upper third covers an about 50 nm wide band; its maximum is 340 nm and of broad surface, which suggests that there is no uniform component involved here (Fig. 9).

The excitation and fluorescence spectra of the crude spermosin fraction reveal that here we are dealing with a mixture of more than one component. It is mainly the fluorescence spectrum that shows a considerable deviation from

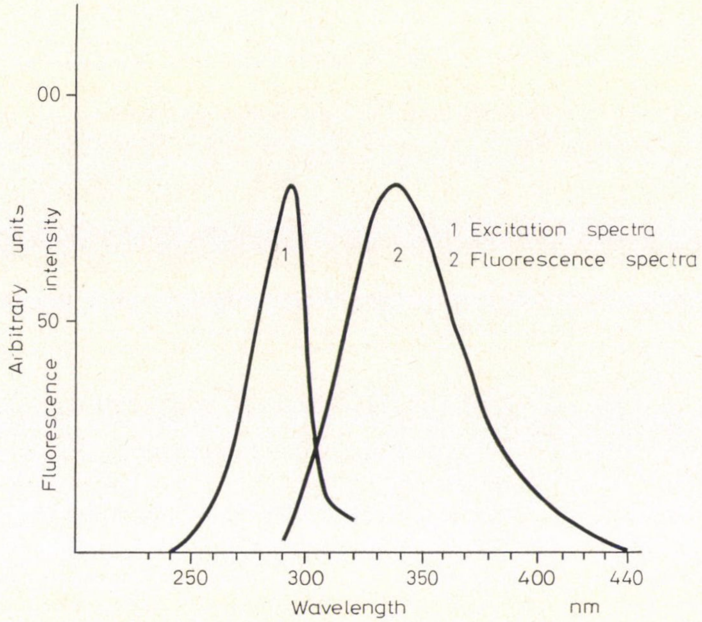

Fig. 9. Excitation and fluorescence spectra of actospermosin after a single purification

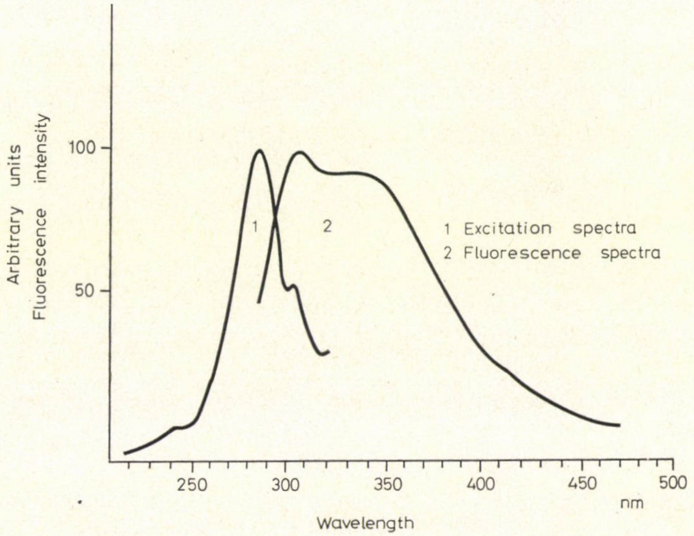

Fig. 10. Excitation and fluorescence spectra of spermosin before chromatography

the spectra of pure proteins in the lower range of the wavelength. The fact that it shows a considerable emission even beyond 470 nm suggests the presence of lipids (Fig. 10).

The spectra of fractions III and IV separated chromatographically and possessing an ATP-ase activity strongly resemble the pure proteins, but a

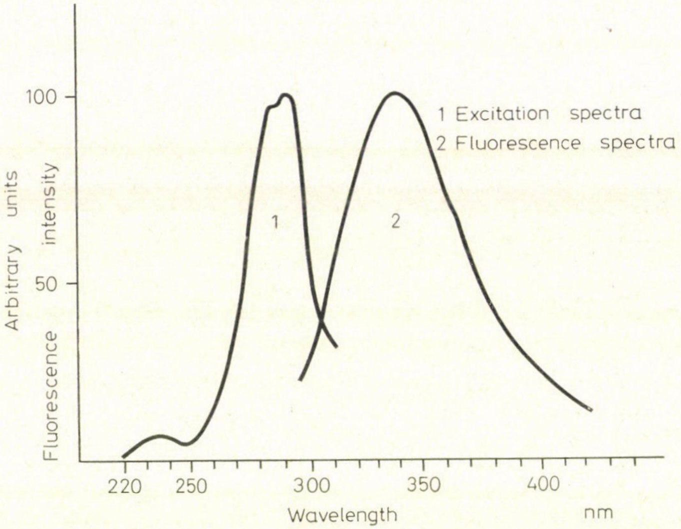

Fig. 11. Excitation and fluorescence spectra of the third fraction of chromatographied spermosin

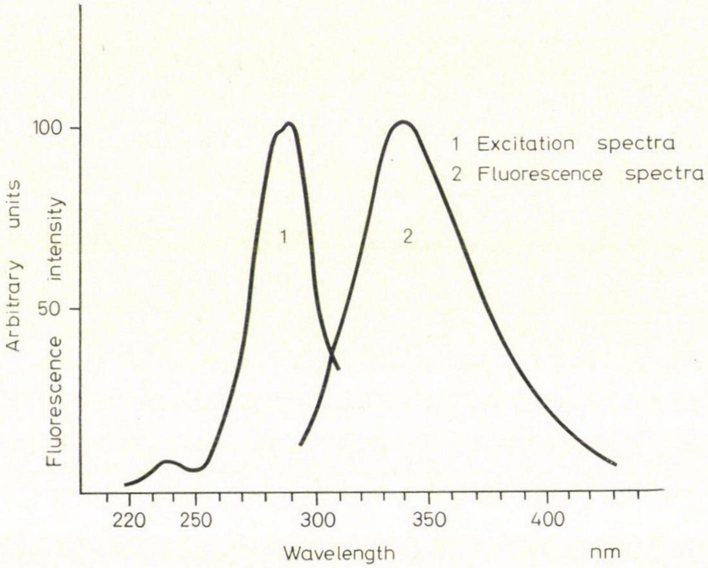

Fig. 12. Excitation and fluorescence spectra of the fourth fraction of chromatographied spermosin

double peak appears in the excitation spectra of both of them which is more expressed in fraction III. Accordingly, the fluorescence spectrum covers a broader surface of the upper third in fraction III than in fraction IV (Figs 11 and 12).

The amino-acid composition of actospermosin and spermosin (peak IV) was determined, and the results are summed up in Table 1.

Table 1

Amino-acid composition of actospermosin and the spermosin fractions IV and V expressed in Mol/100 Mol total amino-acid residues

Amino acid	Actospermosin	Spermosin IV	Spermosin V
	M/100 M	M/100 M	M/100 M
MeSO	2.1	in traces	in traces
Asp	11.0	8.8	7.30
Thr	6.5	1.8	6.70
Ser	9.3	13.5	11.70
Glu	12.9	13.6	16.00
Pro	6.1	2.3	in traces
Gly	9.2	17.7	23.0
Ala	8.0	8.7	8.55
Val	8.7	1.5	2.89
Met	2.0	4.0	3.5
Ile	1.12	2.0	1.10
Leu	7.1	6.5	3.28
Tyr	3.1	3.4	2.12
Phe	2.1	7.4	8.3
Try*			
Lys	8.4	9.9	8.4
His	1.9	3.1	in traces
Me-Lys	1.1	0.76	1.22
Arg	4.6	6.2	5.12

* No identification of tryptophane has been made

The table gives the average data obtained from the amino-acid analysis of actospermosin derived from four separate isolations, and of the chromatographed spermosin fractions IV and V. Both actospermosin and spermosin fractions IV and V contain large quantities of mono-amino-dicarbonic acid (Asp and Glu) like the muscle actomyosin and myosin.

Actospermosin contains approximately M Me-Lys (100 amino-acid residues). In the spermosin fractions Me-Lys is always present; in fraction IV we determined about 0.7, while in fraction V a somewhat higher average — about 1.2 M/100 — for Me-Lys. Although the value of the latter is higher, but — as mentioned before — it contains nucleic acid and lipid too, fraction V is supposedly not the only protein.

The results of chromatographic fractions II and III need repetition. Our distrust is based on the fact that the quantity of fractions II and III varies in the successive preparations, now fraction II, now fraction III shows a larger peak area. We assume that the differences are caused by the fact that the ejaculates originate from 8 or 9 different bulls.

When summarizing our results, we establish that the spermosin originates from the fibrillar system of the tail part of the spermatozoa — from the subfibers, or more precisely from its elementary units, the protofibrils. The spermosin purified by preparative methods is chromatographically heterogeneous, and can be separated into at least 4 fractions exhibiting ATP-ase activity. Of them fractions IV and V show a tendency to form aggregates, while standing artificial filaments like in the myosin and actomyosin systems are produced in them.

It has remained undecided, however, from which of the two filament systems the spermosin fraction originates, or more precisely, we have no evidence whether the protofibrils of the crude spermosin fraction are produced by the outer, the inner or by both filamentary systems.

Heterogeneity is also supported by the excitation and fluorescence spectra of the individual fractions. The bands of the excitation and fluorescence spectra of the corresponding fractions originating from striated rabbit muscle are narrower, and those of fractions before chromatography are more homogeneous, too.

Data obtained with amino-acid analysis show that the actospermosin and the spermosin fractions IV and V are proteins of acidic character, still they greatly differ in amino-acid composition from the actomyosin and myosin originating from the muscles of mammals. We wish to note here that according to the electron-microscopic photos, the actospermosin fraction consists of outer and inner filaments of various length, and of artificial filaments reaggregated from their dissolved substance.

The methylated lysin peak is systematically present in all three fractions examined, and on the basis of comparisons seems to be ε-N-trimethyl lysin. We have tried to prove this statement by the paper and thin-layer chromatography methods of TYIHÁK—VÁGUJFALVI (1970) and TYIHÁK (1973), but the results obtained so far are not sufficiently reliable; much more initial material and the previous separation and concentration of the amino acids are needed.

It is a question more and more frequently raised in the literature that filaments similar to actin occur in mammalian protozoa too (YOUNG—NELSON, 1969), moreover, they can be electron-microscopically observed in preparations isolated from the testicles of such small ainmals as *Nephrostoma saturalis* Loew (Crane fly, BEHNKE *et al.* 1971). In spite of the literary data, so far we have not succeeded in collecting as much "actin fractions" as enabling us to prove their actin character by biochemical methods.

Acknowledgement

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REACTION OF A LITTLE-KNOWN LABIATAE PLANT TO TWELVE PLANT VIRUSES

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Our artificial infection experiments relative to the virus susceptibility of *Ocimum canum* Sims (Family: *Labiatae*) showed that it was locally susceptible to the tobacco rattle virus (R/1 : 2.3/5 : E/E : S/Ne) as well as locally and systematically sensitive to cucumber mosaic virus (R/1 : 1/18 : S/S : S/Ap), potato aucuba mosaic virus (*/* : */* : E/E : S/Ap), potato virus X (R/1 : */6 : E/E : S/Fu), tobacco mosaic virus (R/1 : 2/5 : E/E : S/*) and tobacco ring spot virus (R/1 : 1.8/42 : S/S : S/Ne). During the infection experiments *Ocimum canum* Sims proved to be immune from six viruses (bean [common] mosaic virus: */* : */* : E/E : S/Ap; potato virus M, */* : */* : E/E : S/Ap; potato virus S, */* : */* : E/E : S/Ap; potato virus Y, */* : */* : E/E : S/Ap; radish mosaic virus, R/* : */* : S/S : S/Cl and turnip yellow mosaic virus, R/1 : 1.9/37 : S/S : S/Cl). As a screening host plant *Ocimum canum* Sims can be used for the separation of certain viruses, and is expecially suitable for the identification of cucumber mosaic virus.

Introduction

The experiments in the course of which *Ocimum basilicum* L. belonging to the family *Labiatae* was first found to be susceptible to tomato spotted wilt virus (R/* : */* : S/S : S/Th) began almost three decades ago (Delle Coste–Zabala 1946). According to our present knowledge, *Ocimum basilicum* L. is — in addition — susceptible to the following viruses: potato virus X (R/1 : */6 : E/E : S/Fu), Ladeburg *et al.* 1950), tobacco rattle virus (R/1 : 2.3/5 : E/E : S/Ne, Schmelzer 1957), cucumber mosaic virus (R/1 : 1/18 : S/S : S/Ap, Lovisolo 1958, Horváth 1969), *Lamium* mild mosaic virus (*/* : */* : */* : S/Ap, Lovisolo 1958), tobacco necrosis virus (R/* : */* : S/S : S/Fu, Gigante 1959), alfalfa mosaic virus (R/1 : 1.3/18 : U/U : S/Ap, Lovisolo 1960, 1961), tobacco mosaic virus (R/1 : 2/5 : E/E : S/*, Lovisolo 1960), tomato bushy stunt virus (syn.: *Pelargonium* leaf curl virus, R/1 : 1.5/17 : S/S : S/*, Lovisolo 1960), *Tropaeolum* ring spot virus (syn.: *Nasturtium* ring spot virus, */* : */* : */* : S/Ap, Schmelzer 1960), cherry Pfeffinger disease virus (*/* : */* : */* : S/Ne, Kegler 1960), arabis mosaic virus (R/1 : */41 : S/S : S/Ne, Schmelzer 1962, Klinkowski–Uschdraweit 1968a), cowpea aphid-borne mosaic virus (*/* : */* : E/E : S/Ap, Lovisolo 1966, Lovisolo–Conti 1966), *Petunia* asteroid mosaic virus (syn.: *Pelargonium* leaf curl virus and tomato bushy stunt virus, see before by tomato bushy stunt virus, Lovisolo 1966), raspberry ring spot virus (*/* : */44 : S/S : S/Ne, Kegler 1968), strawberry vein clearing virus (*/* : */* : */* : S/(Ne), Kegler 1968), tomato black ring virus (*/* : */* : S/S : S/Ne, Schmelzer 1963, Klinkowski—Uschdraweit 1968b) and papaw mosaic virus (*/* : */* : E/E : S/*) as well as papaw ring spot virus (*/* : */* E/E : S/Ap, Cook—Milbrath 1971). On the basis of the results of experiments it is further known that *Ocimum basilicum* L. is not only an artifical test plant but also a natural host plant of cucumber mosaic virus (cf. Marini 1955) and alfalfa mosaic virus (cf. Feldman—Gracia 1970). According to the available literary data and our experiences, *Ocimum basilicum* L. was found to be resistant to the following nine plant viruses : beet mosaic virus (*/* : */* : E/E : S/Ap, Lovisolo 1960), cabbage black ring spot virus (syn.: turnip mosaic virus, */* : */* : E/E : S/Ap, Lovisolo 1960), potato virus Y (*/* : */* : E/E : S/Ap, Lovisolo 1960), bean (common) mosaic virus (*/* : */* : E/E : S/Ap, Lovisolo 1966), cowpea mosaic virus (R/1 : 1.1/31 : S/S : S/Cl, Lovisolo 1966), henbane mosaic virus (*/* : */* : E/E : S/Ap, Lovisolo 1966), potato aucuba mosaic virus (*/* : */* : E/E : S/Ap, Lovisolo 1966), *Sorghum* red stripe disease virus (syn.: sugarcane mosaic virus, */* : */* : E/E : S/Ap, Lovisolo 1966) and turnip yellow mosaic virus (R/1 : 1.9/37: S/S : S/Cl, Lovisolo 1966). According to the data of literature, *Ocimum basilicum* L. is primarily susceptible to the isometric viruses (except for cowpea mosaic virus and turnip yellow mosaic virus).

About the virus susceptibility of another less known *Ocimum* species, *Ocimum canum* Sims hardly anything is known. According to our knowledge, *Ocimum canum* Sims is only susceptible to tomato black ring virus (*/* : */*: S/S : S/Ne, Schmelzer 1963), cherry leaf roll virus (*/* : */* :S/S : S/Ne, Schmelzer 1966), poplar mosaic virus (*/* : */* : E/E : S/*, Schmelzer 1966) and alfalfa mosaic virus (cf. Horváth—Beczner 1973a). Considering that our knowledge of the virus susceptibility of *Ocimum canum* Sims is still very incomplete, we carried out investigations on the question whether this plant is suitable for the identification of other viruses and the separation of the different viruses.

Material and Method

In our artifical infection experiments the following viruses and virus strains were used: bean (common) mosaic virus (Horváth 1973), four strains of cucumber mosaic virus (E, R, T and W strains, cf. Horváth—Szirmai 1973, Horváth 1969, Horváth—Beczner 1973b, Skiebe—Schmelzer 1967), potato aucuba mosaic virus (Horváth 1972a), two strains (Bi and Fo) of potato virus M (*/* : */* : E/E : S/Ap, Horváth—De Bokx 1971), two strains (L and Yss) of potato virus S (*/* : */* : E/E : S/Ap, Horváth 1972b), potato virus X (Horváth—Beczner 1968), normal strain and veinal necrosis strain of potato virus Y (Horváth 1966, 1967), two strains (HZ and H7) of radish mosaic virus (R/* : */* : S/S : S/Cl, Štefanac—Mamula 1971, Horváth *et al.* 1973), U1 strain of tobacco mosaic virus (Siegel—Wildman 1954), tobacco rattle virus (Horváth 1973), tobacco ring spot virus

(Horváth 1973) and two strains (Y65 and H4) of turnip yellow mosaic virus (Mamula 1968, Juretić *et al.* 1973).

Before the inoculation the young *Ocimum canum* Sims plants used in the experiment were dusted with carborundum (mesh 500). Tissue saps containing viruses were diluted before inoculation with 0.15 *M* pH 7.0 buffer at a ratio 1 : 1. After inoculation performed with the carborundum-spatula technique the infected plants were sprinkled with tap water. From inoculated and non-inoculated or subsequently developed leaves of *Ocimum canum* Sims plants remaining symptomless after inoculation, viruses were reisolated on the third or fourth week to susceptible host plants: *Brassica rapa* L. var. rapa (radish mosaic virus and turnip yellow mosaic virus), *Gomphrena globosa* L. (potato virus X), *Nicotiana glutinosa* L. (potato aucuba mosaic virus), *Nicotiana tabacum* L. cv. Samsun (tobacco rattle virus), *Nicotiana tabacum* L. cv. Xanthi-nc (tobacco mosaic virus and tobacco ring spot virus), *Phaseolus vulgaris* L. (bean [common] mosaic virus and tobacco ring spot virus), *Solanum demissum* Lindl. A6-hybrid (potato virus Y) and *Tetragonia tetragonoides* (Pall.) O. Ktze. (cucumber mosaic virus). Before the reisolation of viruses the inoculated leaves of *Ocimum canum* Sims were surface-sterilized with a 2 per cent NaOH solution, then washed repeatedly with a sharp jet of tap water, and from the leaves tissue sap was made in the usual way. We attempted to demonstrate the presence of potato virus M and potato virus S by using the serological method of agglutination (cf. Horváth 1971). In the serological tests both the inoculated and the non-inoculated, newly developed leaves of *Ocimum canum* Sims were taken into consideration.

Results

On the basis of our experimental results it could be established that one of the twelve viruses included in the experiment (tobacco rattle virus) caused only local symptoms, while five of them (cucumber mosaic virus, potato aucuba mosaic virus, potato virus X, tobacco mosaic virus and tobacco ring spot virus) caused local and systemic diseases or symptoms in the inoculated *Ocimum canum* Sims plants (Table 1). The examined *Ocimum canum* Sims plants proved to be resistant to six viruses (bean [common] mosaic virus, potato virus M, potato virus S, potato virus Y, radish mosaic virus and turnip yellow mosaic virus). The most severe systemic symptoms were caused by various strains of cucumber mosaic virus (Fig. 1). From a symptomatological point of view we think it desirable to mention that about four weeks after the infection intensive stem and foot necrosis appeared in each case on the inoculated *Ocimum canum* Sims plants (Fig. 2). Investigations were made to find out whether the above symptoms are in any causal relation with the virus infection. Tissue saps were prepared from the stems and feet of plants showing stem- and foot-necrosis. With the tissue sap obtained diagnostical test plants were inoculated (see Material and Method). Several days after the inoculation severe symptoms appeared on the test plants inoculated with the tissue sap obtained from the stem and foot of *Ocimum canum* Sims plants infected earlier with tobacco rattle virus and tobacco mosaic virus. From this we have drawn the conclusion that the symptoms of stem- and foot-necrosis are in connection with the virus infection. This theory is, however, inconsistent with the negative results, namely, that the viruses included in the experiment (with the exception of tobacco rattle virus and tobacco mosaic virus) could in no case be demonstrated in test plants inoculated with the tissue sap obtained from the necrotic stems and feet of

Table 1

Reaction of Ocimum canum Sims to several plant viruses

Viruses	Symptoms*	Reisolation of viruses or serological results**
Bean (common) mosaic virus	IL: no symptoms NIL: no symptoms	IL: negative NIL: negative
Cucumber mosaic virus	IL: no symptoms NIL: severe mosaic and leaf deformation; mosaic and deformation on the axillary shoots (Fig. 1.)	IL: positive NIL: positive
Potato aucuba mosaic virus	IL: necrotic spots NIL: no symptoms	IL: positive NIL: positive
Potato virus M	IL: no symptoms NIL: no symptoms	IL: no reaction NIL: no reaction
Potato virus S	IL: no symptoms NIL: no symptoms	IL: no reaction NIL: no reaction
Potato virus X	IL: necrotic spots NIL: no symptoms	IL: positive NIL: positive
Potato virus Y	IL: no symptoms NIL: no symptoms	IL: negative NIL: negative
Radish mosaic virus	IL: no symptoms NIL: no symptoms	IL: negative NIL: negative
Tobacco mosaic virus	IL: necrotic spots and mosaic NIL: mosaic	IL: positive NIL: positive
Tobacco rattle virus	IL: necrotic spots NIL: no symptoms	IL: positive NIL: negative
Tobacco ring spot virus	IL: mosaic NIL: mosaic on the leaves and on the axillary shoots	IL: positive NIL: positive
Turnip yellow mosaic virus	IL: no symptoms NIL: no symptoms	IL: negative NIL: negative

Abbreviations used: * IL: inoculated leaves; NIL: non-inoculated leaves or subsequently developed leaves of the infected *Ocimum canum* Sims plants. ** Serological results by potato virus M and potato virus S

plants infected with the different viruses. Experiments were further carried out to find out whether the necrotic diseases of stems and feet of *Ocimum canum* Sims plants were not caused by some soil fungus or bacterium. According to the results of experiments only saprophytic bacteria could be isolated from the necrotic stem and foot tissues. Considering that both viruses could be re-isolated also from the roots of *Ocimum canum* Sims plants inoculated with tobacco rattle virus and tobacco mosaic virus we are inclined to think that the symptoms of stem- and foot-necrosis can be explained by the presence of saprophytic bacteria rather than by virus infection.

Discussion

On the basis of our experimental results it can be established that in addition to tomato black ring virus, cherry leaf roll virus, poplar mosaic virus (cf. Schmelzer 1963, 1966) and alfalfa mosaic virus (Horváth—Beczner

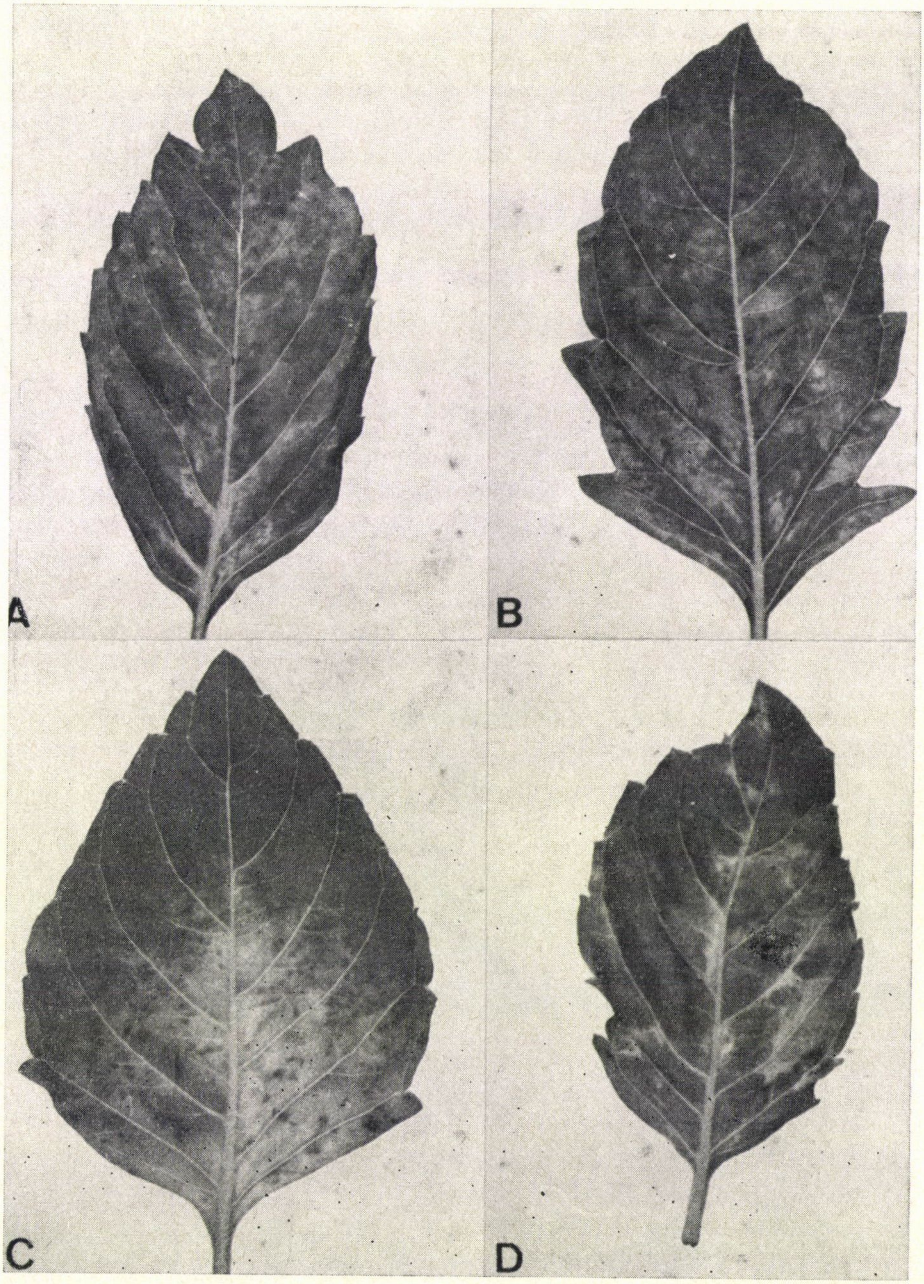

Fig. 1. Symptoms caused by various strains of cucumber mosaic virus on leaves of *Ocimum canum* Sims. A: E-strain; B: R-strain; C: T-strain and D: W-strain of cucumber mosaic virus

1973a) *Ocimum canum* Sims is also susceptible to cucumber mosaic virus, potato aucuba mosaic virus, potato virus X, tobacco mosaic virus, tobacco rattle virus and tobacco ring spot virus. Considering that *Ocimum canum* Sims is resistant to infection by bean (common) mosaic virus, potato virus M, potato virus S, potato virus Y, radish mosaic virus and turnip yellow mosaic virus, it can be used as a screening plant for separating certain viruses. It is noteworthy that *Ocimum canum* Sims, unlike *Ocimum basilicum* L., is susceptible to potato aucuba mosaic virus. However, as for their responses to other viruses studied so far, the two *Ocimum* species proved to be similar. According to the results of experiments *Ocimum camum* Sims is susceptible to five isometric and five elongated plant viruses, and resistant to one isometric and five elongated viruses.

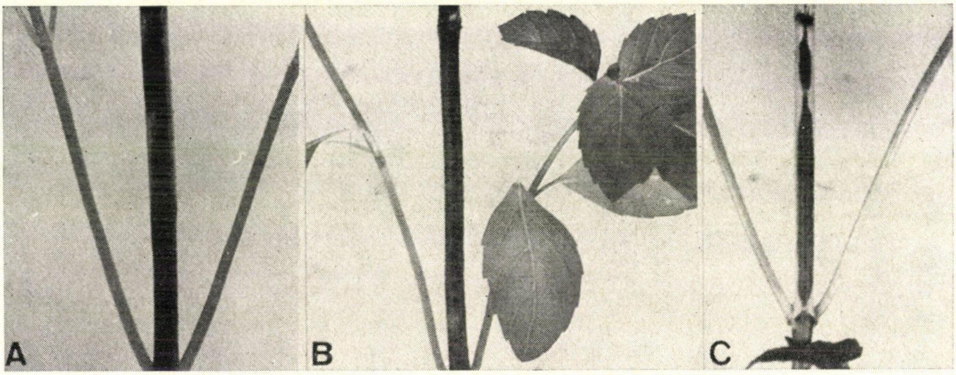

Fig. 2. Symptoms of stem necrosis caused by saprophytic bacteria on *Ocimum canum* Sims. plants infected by various viruses. A: potato virus X; B: tobacco mosaic virus, and C: tobacco rattle virus infected plants

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AERIAL ENVIRONMENT AND TOLERANCE OF POLYGONATUM ODORATUM (MILL.) DRUCE IN NATURAL COMMUNITIES

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The author studied the net photosynthetic reactions of *Polygonatum odoratum* in natural communities of the Buda Mountains (Hungarian Central Mountains). In the system studied the many years old stands ("polycormons") showed the largest extension and individual number in the *Brachypodium* type of *Orno-Quercetum*, while on the steppe meadow of extreme illumination and at the poorly lighted habitat of *Vicia sparsiflora* type of the *Orno-Quercetum* only a few individuals occurred. The author characterizes the light climate of the forest habitats by the frequency distribution of the illumination-intensity values and examines the light curves of *Polygonatum* in this context. As regards the daily trends the gross photosynthesis of the steppe meadow plants counterbalances the respiration loss to 32—33 °C and — unlike the other types — is characterized by an occasionally considerable photosynthetic activity recurring in the afternoon. The linear phase of the light curve of forest plants growing at habitats of poor light conditions is less steep than of those developing at better illuminated *Brachypodium* habitats, also the total chlorophyll content per unit area is smaller in the former, and the most frequent site-illumination values are utilized by them with ten times lower efficiency than by plants growing at the *Brachypodium* habitats. The poor adaptation to shade of the species may be an ecological limiting factor.

Introduction

For photophilous forest plants the light deficiency at the base of the forest is a factor limiting their spreading. Although this is a generally known fact, there are but very few studies available which adequately assess the habitat conditions and specific properties relative to the nature of limitation and tolerance.

In the course of our observations among the species taken in consideration *Polygonatum odoratum* seemed for various reasons the most suitable for investigations of this kind.

Material and Methods

The investigation took place on the Remete-hill, in the Buda Mountains. For the detailed description of the site and vegetation see Précsényi—Fekete—Szujkó-Lacza 1967, Szujkó-Lacza—Fekete 1971. The limestone vegetation — first of all on the hillside — consists of oak-wood rich in xerothermophilous submediterranean elements *(Orno-Quercetum)* with *Quercus pubescens* domineering in it, of a slackening open karstic bush forest *(Ceraso-Quercetum)* at the edge of the karst plateau, and of a steppe meadow *(Diplachno-Festucetum sulcatae)*, (Fig. 1) among the tree groups.

The individual *Polygonatum odoratum* test plants were selected from habitats with different light (and heat) conditions. This paper presents some major results of a series of measuring performed on plants obtained from three different habitats. These habitats as specified by the name of the plant community and the dominant species of the community were: *Diplachno-Festucetum*, hereinafter called steppe meadow (dominant species, competitors of soil moisture and nutrient: *Agropyron intermedium*, *Festuca valesiaca*, in autumn *Diplachne serotina*); *Orno-Quercetum Brachypodium pinnatum* facies, this species too is a strong soil competitor of *Polygonatum odoratum* (hereinafter called: *Brachypodium* habitat); *Orno-Quercetum Vicia sparsiflora* facies which, in the first place, gives an intensive shading to the test plant (hereinafter called: *Vicia* habitat).

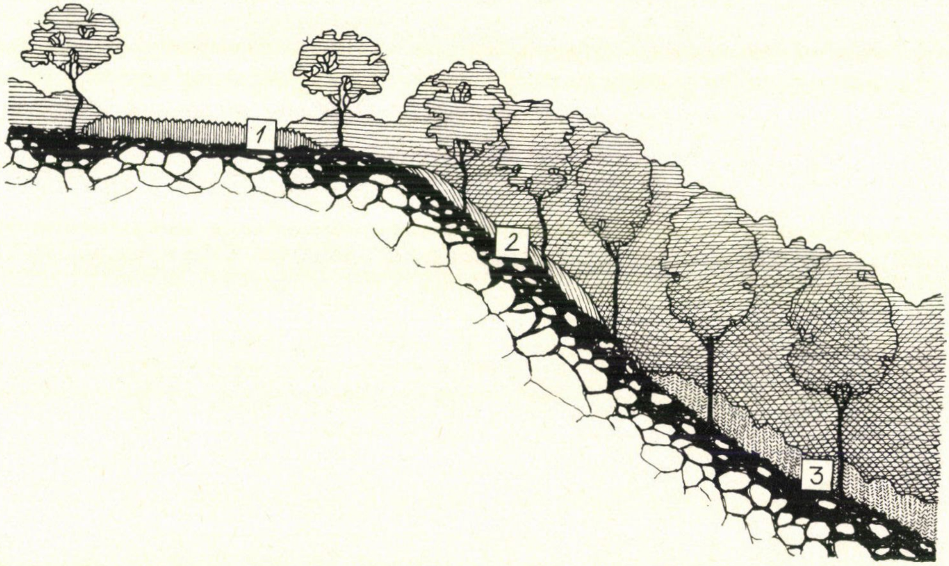

Fig. 1. The three habitats of *Polygonatum odoratum* and their location. 1. *Diplachno-Festucetum sulcatae* (steppe meadow); 2. *Orno-Quercetum Brachypodium pinnatum* habitat; 3. *Orno-Quercetum Vicia sparsiflora* habitat

From the point of view of light ecology the steppe meadow represents one of the extremities; here during the summer months in clear weather, *Polygonatum* is exposed to an illumination of 50 000 lux or more over a period even exceeding five hours a day. A high fluctuation of illumination and intensive warming up are characteristic of this site.

The *Vicia* habitat which is characterized by a closed foliage and shrub stratum justifed by the ecological aspects of the soil represents the other extremity as regards the intensity of illumination. It is an extreme habitat since no *Polygonatum odoratum* individuals can practically be found in a light climate poorer than that.

The *Brachypodium* habitat — while representing a transition between the two former habitats — is naturally closer to the other forest *(Vicia)* habitat as regards basic illumination (TRANQUILLINI 1960) and temperature.

To assess the light conditions characteristic of the life cycle of *Polygonatum odoratum* from the end of April till the middle of August 1972, photometric data were recorded three times a month with a lux meter every hour from 6 a. m. to 6 p.m. Photometry was performed holding the selenium cell of the lux meter immediately above the plants selected for photosynthesis measurements. The days of photometric measuring were the following: 24 April; 3 May; 14 May; 23 May; 4, 15, 23 June; 4, 16, 20 July; 5, 15, 16 August. These days give the illumination values of different weather conditions. The 169 illumination values thus observed at each habitat are grouped in classes of a thousand lux, and their frequency is given as a percentage. The light climates of the *Brachypodium* and *Vicia* habitats are shown in Fig. 3.

The location of the three habitats is shown by Fig. 1. The habitats are at a distance of 30—40 m from one another.

From each of the stands developed at the three habitats — the smaller or larger groups of shoots (groups of plants) of *Polygonatum odoratum* can be regarded as younger or older vegetative reproduction units, stands, or "polycormons" (PÉNZES 1960) — a vigorous shoot was chosen in 1972 for the purpose of investigations carried on over three months. When the series of measuring was completed, a 3—4-years old part could be seen on the rhizomes.

The CO_2 assimilation was measured with a portable apparatus working on the basis of the principle of conductometry (VOZNESENSKII 1971). The apparatus measures the CO_2 content of 0.5 l. air by an about 3 minutes perfusion into 0.02 n KOH, with a preciseness of 0.01 mg. The difference in CO_2 concentration between the open air and the phyllosphaera gave the value of net assimilation. The values of illumination on the leaf were measured, noticed and the temperature values inside the casette were fixed during the 3—3.5 minutes of perfusion. When measuring the respiration, we covered the casette with a bag made of lightproof black textile. Three terminal leaves of each of the 5—6 leaved plants were placed in the casette after their leaf area had been recorded.

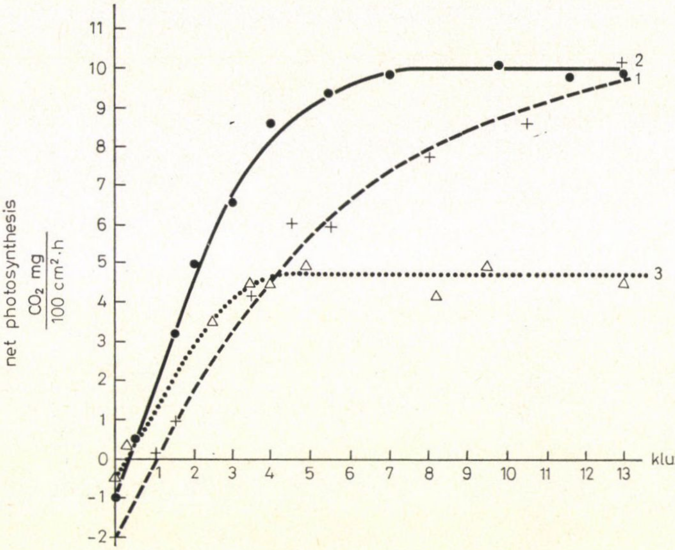

Fig. 2. The light curve of net photosynthesis in *Polygonatum odoratum* at 21 °C. 1: steppe meadow-, 2: *Brachypodium*-, 3: *Vicia* habitat

In August the plants were dug out with large earth-balls, put into pots and placed in the phytotron (in the Botanic Garden of the Department of Botany of the Szeged University). The light curves were determined by means of the apparatus described above on 22—24 August at a temperature of 21 °C (the temperature of the phytotron and the casette of the apparatus, respectively), relative humidity of 50—60 per cent and various intensities of illumination between 0 and 13000 lux.

The quantity of the chlorophyll components was determined according to ARNON (1949).

Results

The light curves of net photosynthesis determined in the phytotron are shown by Fig. 2. The curves were drawn with the highest photosynthesis values observed at the same intensity of illumination taken in consideration.

At the highest (13000 lux) intensity of illumination used the light curve of plants growing in the steppe meadow had not reached its peak; its shape was similar to that of the usual light curve of sun-grown plants.

The light curves of plants obtained from the *Vicia* and *Brachypodium* habitats, respectively, are compared in Fig. 3. which, at the same time, represents the summarized result of light measurements taken at the individual habitats (MALKINA 1970). Light saturation sets in around 5000 lux at the *Vicia*, and 8000 lux at the *Brachypodium* habitat under the given conditions. In the case of plants obtained from the *Brachypodium* site the peak of the light curve is twice as high as that of the former ones. At the same time, the ascending branch (linear phase) is steeper as well.

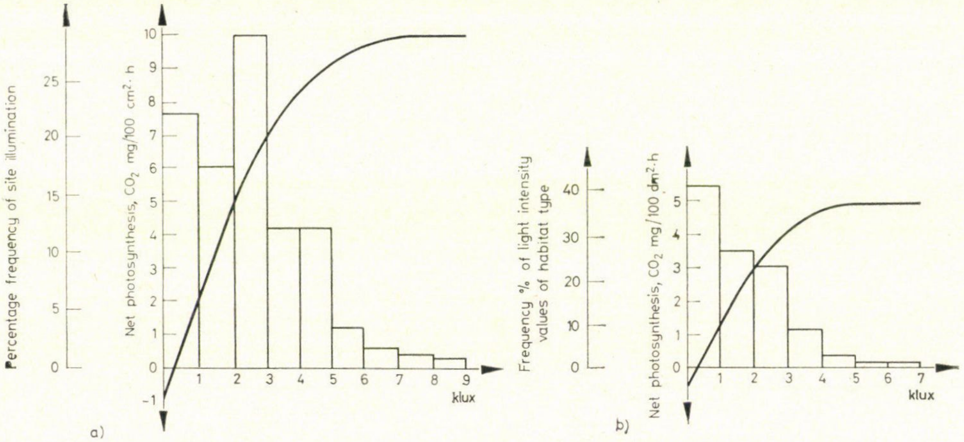

Fig. 3. The light curve of net photosynthesis in *Polygonatum odoratum* in *Brachypodium* (a), in *Vicia* (b), habitat and the percentage frequency distribution of the intensity of site illumination

Of all illumination intervals values between 0 and 1000 lux occur with the highest frequency — of about 40 per cent — at the *Vicia* site. The probability of as low values as that occurring at the *Brachypodium* site is much less (Fig. 3.); the most probable value is 2—3000 lux here. The frequency of light intensities above 3000 lux is some 30 per cent at the latter habitat, while at the *Vicia* site only 11 per cent; within this range the plants of the *Brachypodium* site incorporate nearly twice as much CO_2 per unit time as the plants of the other habitat.

Chlorophyll contents involved in light utilization are given in Table 1.

It is, naturally, not only the light (and light – chlorophyll complex, respectively) that determines the CO_2 balance at the genuine habitat. Of the various modifying factors, temperature, its daily trend and the response of

Table 1

Total chlorophyll content in Polygonatum odoratum plants originating from three different habitats, and the ratio of the components

Habitat	Total chlorophyll mg/100 cm^2	Chlorophyll a/b
Steppe-meadow	1.065	2.16
Brachypodium	2.614	2.43
Vicia	2.037	2.21

plants to it may be decisive. Table 2 mostly presents 12 hours' measuring results characteristic of the three habitats, with 2 and 3 days of different mean temperature taken in consideration for *Brachypodium* and *Vicia*, respectively. The soil was sufficiently supplied with water in all six cases.

To demonstrate the activity of the steppe-meadow plant, a typical summer day was chosen. The daily balance was positive in spite of the fact that the temperature of the leaf (casette) was higher than 30 °C over more than seven hours. The loss at noon was compensated for by the higher positive values measured at 6—7 a.m. and 4—5 p.m.

It is this latter photosynthetic activity recurring in the afternoon that characterizes this type in comparison with the other two.

At the *Brachypodium* site the plants utilize the higher photosynthetic capacity appearing on the 21 °C light curve mostly in the morning hours in June — July and August. Depression at noon, a negative or zero net photosynthesis was often measured during these months. Time had an essential part in these trends, and in many cases the gas exchange did not turn into positive again, even in the afternoon. Above 25 °C a CO_2 loss of 5—6 mg was often measured in the dark, and the fact that as high a negative value as that occurred in the light too, suggests that on hot summer days the photosynthetic activity often stops in this plant, while the respiration is of considerable degree.

On the other hand, the balance of May is highly favourable. No depression at noon could be demonstrated at that time. At a temperature around 20—21 °C (which is frequent in May) the light curve well-adapted to the habitat of *Brachypodium* holds good, and the daily balance of net photosynthesis shows a high positive value.

At the *Vicia* site noon depression of a degree similar to that of the other forest site was not observed. Even the values obtained in the dark only suggest a respiration of 1—2 mg/100 cm^2/h. The daily trend of 7 July characterizes a cloudless day with a moderately warm temperature; the balance of net photosynthesis during day-time (the average of 12 measurements) is then nearly the same as in the steppe-meadow or at the *Brachypodium* site. On cloudy days —

Table 2

Daily trends in the net photosynthesis of Polygonatum odoratum at various habitats. 1: casette temperature, °C; 2: illumination, Klux; 3: mg $CO_2/dm^2/h$.

Habitat	Steppe-meadow			Brachypodium					
Date	10 October 1972			8 June 1972			5 August 1972		
	1	2	3	1	2	3	1	2	3
time h									
6	21	6.0	7.5	22	1.5	2.5	16	0.6	0.0
7	22	8.0	2.1	23	2.0	4.0	17	0.8	1.2
8	24	10.0	2.1	24	2.0	5.2	17	0.9	2.4
9	27	16.0	1.4	25	2.5	6.3	17	1.2	5.8
10	32	25.0	0.5	25	11.0	1.9	20	7.0	8.1
11	41	50.0	—1.4	25	4.0	1.9	20	1.8	0.6
12	41	50.0	—3.6	25	5.0	3.8	23	25.0	—2.4
13	39	50.0	—6.3	27	4.2	— 3.5	20	1.9	—1.5
14	35	50.0	—3.2	28	4.7	—13.3	19	7.0	1.5
15	34	47.0	—2.9	26	1.8	— 2.5	18	0.6	—1.4
16	33	18.0	2.8	25	1.6	— 0.8	19	0.8	1.3
17	27	7.0	6.7	25	0.8	1.2	18	0.6	—0.2
18	23	3.0	2.0	25	0.4	0.6	18	0.4	—0.4

Habitat	Vicia								
Date	7 July 1972			23 May 1972			6 August 1972		
	1	2	3	1	2	3	1	2	3
6	19	0.8	1.1	19	0.2	—0.3	17	0.8	1.3
7	20	1.0	2.5	20	0.3	—0.3	18	1.0	2.5
8	20	1.4	1.6	21	0.4	—0.2	18	1.3	3.5
9	23	2.0	—0.6	21	0.6	1.0	19	1.7	3.5
10	27	3.7	—0.6	22	0.6	1.0	20	4.3	6.8
11	28	3.5	0.0	23	0.9	1.0	23	2.9	4.9
12	33	25.0	0.0	24	1.0	0.8	27	10.0	0.0
13	32	3.5	3.3	25	1.4	0.5	24	3.0	1.0
14	28	2.0	4.8	24	1.2	0.8	23	2.7	2.5
15	27	1.8	1.6	23	0.8	0.6	23	8.5	0.7
16	26	0.9	0.2	23	0.3	—0.6	22	1.7	—0.9
17	25	0.8	0.0	22	0.2	—0.6	21	0.9	—0.3
18	23	0.4	0.0	21	0.2	—0.8	20	0.4	0.0

if only with a thin cover of clouds — the illumination of the site is sharply reduced, as e.g. on 23rd May. On days like that — which are rather frequent — the 12 hours total of net photosynthesis is very low, only 2—3 mg.

The paper further presents the measurement series of an August day; however, the high values of illumination are not characteristic of the site (they were brought about — and the foliage opened, respectively — as the result of a nearby tree falling shortly before). The high positive balance of the day is not characteristic either.

At the three habitats discussed *Polygonatum odoratum* shows differences not only in the ecology of photosynthesis, but also in the life cycle of the individual plants and the extension, development and dynamics of the polycormons. These differences can by and large be summed up in the following.

The steppe-meadow plant is characterized by the typical light curve of a sun-grown plant. Up to a temperature of 32—33 °C — at high light intensites and with a sufficient water supply — the respiration loss is compensated for by the gross photosynthesis. As for these properties, it is thus well-adapted to the extreme habitat. Since in June, with a deficient soil moisture content, low values of net photosynthesis were often measured throughout the day (and high respiration values), it is probable that for this type of steppe-meadow plant water often represents a minimum factor. The generative way of reproduction is important; of 100 plants (obtained by digging up smaller adjacent spots and separating the underground parts) 8 were found to be first-year rhizomes at the stage of green shoot, that is, plants developed with all certainty from seed. The older rhizome parts are quickly destroyed, that is, their nutrient content is used up; not a single rhizome older than four years has been found. Migration by rhizomes is, otherwise, limited in the shallow rendzina soil interwoven mainly with the roots of *Graminea* species.

The light curve of plants growing at the *Brachypodium* site is — as for its plateau — an intermediary type; at 21 °C light saturation sets in around 8000 lux. As regards the ecology of photosynthesis it is thermosensitive: at high temperatures considerable depressions occur. The plants sometimes show signs of mortality: leaves with yellowing or drying edges and decaying shoots in the middle of the summer; and in the soil rhizomes not developing aboveground shoots can often be found that year. In spite of all this, one of the phytocenological centres of the species is represented by this habitat, first of all as regards individual density. The strong individuals reach the stage of fruit ripening and may thereby reproduce green seedlings (6 per cent of the plants develop from seed), so the individuals of the polycormon are joined by ones produced in a generative way.

The plants of the *Vicia* habitat reach the plateau of the light curve of net photosynthesis at 5000 lux. When comparing the plants of the two sites in relation to the harmony of the light curve and light environment, we find the most essential differences in the most frequent site-illumination value and its photosynthetic utilization. At the *Vicia* habitat in the case of the most frequent — 0 – 1000 lux — illumination interval the light curve is of very low efficiency. The plant of the *Brachypodium* habitat — on the other hand — produces ten times as much as the former with 2—3000 lux occurring the most frequently (see Fig. 3.). In the low range of illumination the light curves of plants originating from the *Brachypodium* habitat are the steepest, besides the plateau is high at the intensities of illumination too; of the two forest sites this one has by all means the more favourable light curve (NICIPOROVIC 1968).

It is noteworthy that the total chlorophyll content per unit area of plants living in the *Vicia* habitat is reduced in comparison to those of the *Brachypodium* (Table 1), although in shade-grown plants the chlorophyll content is expected to be high. At sites poor in light the species in many cases enhance the light absorption by increasing their chlorophyll content, therefore in the initial linear phase of the light curve the chlorophyll concentration is a so-called "weak light factor" (GABRIELSEN, 1960). The relatively low inclination in the initial phase of the light curve at the *Vicia* habitat can be well explained by the lower total chlorophyll content. The a/b ratio of chlorophyll is somewhat — though not essentially — lower.

Thus, when placed under poor light conditions *Polygonatum odoratum* — adapted originally to higher intensities of illumination — secondarily adapts itself insufficiently to the lack of light. The imperfect adaptation of photophilous plants was reported by DAXER (1934) in relation with *Zea mays* and *Anemone silvestris*.

The flexible habit of *Polygonatum odoratum* plants only growing in smaller scattered groups at the *Vicia* habitat and very seldom flowering and producing fruits, indicates too that it is a matter of ecological limitation here, which prevents the plant from spreading towards deeper shade.

Experiments carried on at present with the aim of determining the photosynthetic reaction of exchanging, transplanting the plants of the individual habitats are intended to find out the extent to which the above outlined reactions are fixed.

Acknowledgement

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EFFECT OF THE TIME OF TRANSPLANTATION ON DRY-MATTER PRODUCTION AND LIGHT-ENERGY UTILIZATION IN TOMATO

By

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The time of transplantation influences the dry weight and the course of dry-matter accumulation. In the case of early (May) transplantation the dry weight of both tomato varieties studied — "Kecskeméti konzerv" and "Kecskeméti törpe" — increased for three or four months, then slightly decreased (part of the leaves fell). With a late transplantation (June–July) the increase of dry weight lasts for four or five months, but, the later the transplantation, the lower the dry weight of plants. In both varieties fruit production is the highest in the case of early transplantation. The variety "Kecskeméti konzerv" is more reactive to the time of transplantation than the variety "Kecskeméti törpe." The total carbohydrate concentration is generally higher in the variety "Kecskeméti konzerv" than in the "Kecskeméti törpe", while the total nitrogen concentration is nearly identical in the two varieties. Energy utilization is different in the two varieties. This difference can be brought into connection with the morphological properties and stand structure (stand height, density, leaf area) of the two varieties. It is stand density rather than leaf area that determines the energy utilization. The optimum leaf area index is about 0.3 in the variety "Kecskeméti törpe" and 0.6 in the "Kecskeméti konzerv".

Introduction

Under field conditions 1—5 per cent of the light energy is utilized in the organic matter production of plants (Rabinovitch—Govindjee 1969, Máthé 1971). Papers published report on different results obtained with agricultural crops too in different parts of the world (Gaastra 1958).

However, a lot of basic information is missing as to the conditions under which agricultural crops show a maximum photosynthetic light-energy utilization.

Material and Methods

Plants were transplanted in a random block design (Snedecor 1956) at four different times. Two tomato varieties — "Kecskeméti konzerv" and "Kecskeméti törpe" — were used in the experiment. Attention was called to these two varieties by the Agricultural Research Institute of the Danube–Tisza Mid-Region too (Hatalyák 1970). Seedlings were raised in hot-beds. Seedlings were transplanted into the experimental plots in the Botanical Garden of the József Attila University a month after sowing. Between the times of sowing and transplanting there were intervals of 15 days; the first sowing was carried out on 15 April 1969, the first transplantation on 15 May 1969.

The intensity of illumination in and above the plant stand was measured with a lux meter. Measuring took place every half-hour from sunrise to sunset on two occasions in the plant stands of both varieties. When compared to light intensity above the plant stand (100 per cent,) the intensity of illumination was approximately 60 per cent in the stand of "Kecskeméti konzerv" and 70 per cent in "Kecskeméti törpe". Daily average temperature in the "Kecskeméti konzerv" stand was about 2.0 °C, and in the "Kecskeméti törpe" 1.4 °C lower than that measured above the bare soil at the same height.

The first examination was performed 3, the second 5 months after the first transplantation. Beside calculating the energy utilization we made an analysis of the dry matter too. The total carbohydrate (DUBOIS *et al.* 1956) and total nitrogen (KELLEY *et al.* 1946) concentration was determined in the stem and leaf.

Examinations were performed with 10 plants per replication on both occasions. In addition plant height and leaf area were also measured. Energy utilization (E_h) was computed after MURATA *et al.* (1968) by means of the following equation:

$$E_h = \frac{100 \times \text{dry weight of plant (g)} \times 4000 \text{ (cal)}}{\text{light energy available}} \%$$

In the stands of "Kecskeméti konzerv" and "Kecskeméti törpe" light energy available means 60 and 70 per cent, respectively, of the total light energy, since according to light measuring in the plant stand — as described above — the intensity of illumination compared to that above the plant stand was 60 and 70 per cent, respectively. Radiation energy was measured at the Szeged Meteorological Station at a distance of about 5 km from the experimental area.

Results

Table 1 summarizes the dry weights of plants transplanted at various times as determined in the first and second examination.

Table 1

Dry weights of plants transplanted at different times, as determined in the first and second examination
(Dry weight: g)

Time of transplantation	First examination		Second examination	
	Kecskeméti konzerv	Kecskeméti törpe	Kecskeméti konzerv	Kecskeméti törpe
First	107.9	93.8	100.2	71.0
Second	79.2	60.6	84.2	61.0
Third	22.8	16.5	58.0	32.6
Fourth	6.8	3.5	41.2	32.6
S.D. 5%	15.6	7.2	3.7	1.8

In both varieties the highest dry weights were obtained with plants transplanted on the first occasion. The time of transplantation influences the dry weights of plants between the first and second examination differently. The dry weight of plants — especially of "Kecskeméti törpe" — first transplanted decreased in the period between the two examinations, while that of plants transplanted later increased. The later the time of transplantation, the higher the relative increase of dry weight. In an absolute value, however, dry weight decreased as a function of later transplantation, almost to the

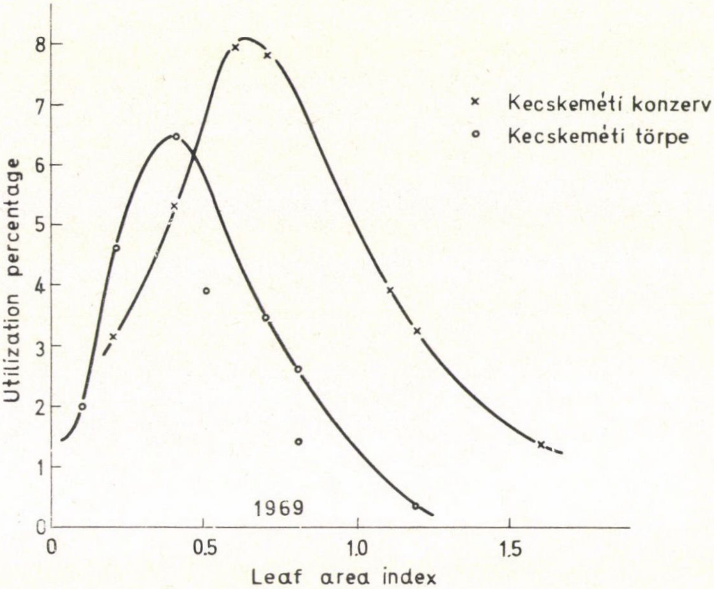

Fig. 1. Relationship between energy conversion and leaf area index

same extent in the two varieties. Correlation between the time of transplantation and change of dry weight resulted partly from the fact that during the ontogenetic cycle dry-weight change per unit time initially showed an increasing, while later a decreasing tendency. The trend of the dry weight was further influenced by the environment, since in the case of plants transplanted at different times the same climatic factors acted on plants of different age. The extent of the effects of these internal and external factors cannot, however, be analysed on the basis of field experiments alone. With plants transplanted on the first occasion, the decrease of dry weight found in the second examination was caused by some of the leaves having been shed between the two examinations.

In plants transplanted on the first and second occasion dry weight increased for 3—4 months, while in those transplanted on the third and fourth occasion for 4—5 months.

Table 2 contains the dry berry weight of plants transplanted at different times, as found in the second examination.

From the dry weight of berries one may conclude on "Kecskeméti konzerv" reacting to the time of transplantation to a greater extent than "Kecskeméti törpe". In both varieties transplantation carried out in May resulted in the highest yield.

The dry-matter production of plants is highly influenced by the environmental factors including the light conditions. A higher dry-matter production

Table 2

Dry weight of berries per plant (g)

Time of transplantation	Kecskeméti konzerv	Kecskeméti törpe
First	49.8	47.1
Second	43.8	43.2
Third	22.2	18.8
Fourth	5.7	15.0

occurring as a result of early transplantation can be attributed first of all to the joint effect of temperature and light. Lower temperatures — around 20 °C — result in a greater dry-matter increase. In this context we may refer to the well-known fact that the optimum temperature of photosynthesis is lower than that of respiration.

Besides, in the case of early transplantation, there develops a larger root system, more leaves and larger leaf area, which are also in correlation with the dry-matter production (WATSON 1952, BONDE 1955, SOMOS 1969, NUERNBERGK 1966, NICHIPOROVICH 1969). The relationship between berry yield on one hand, and leaf number and leaf area on the other, is confirmed by our investigations too.

Tables 3 and 4 present data obtained in the first examination on total carbohydrate and total nitrogen contents in the stems and leaves of plants transplanted at various times.

The total carbohydrate content in the stem and leaf decreases in both varieties as a function of the dry-weight decrease, while the total nitrogen content increases.

It the course of earlier investigations we found — both under field and controlled conditions — that the quality of organic matter developing

Table 3

Total carbohydrate content (gamma/mg)

Time of transplantation	Kecskeméti konzerv		Kecskeméti törpe	
	stem	leaf	stem	leaf
First	364	267	324	169
Second	196	133	179	120
Third	153	150	148	118
Fourth	116	132	130	125

Table 4

Total nitrogen content (gamma/mg)

Time of transplantation	Kecskeméti konzerv		Kecskeméti törpe	
	stem	leaf	stem	leaf
First	15	30	14	29
Second	16	30	17	29
Third	25	36	20	35
Fourth	28	43	23	43

in the plants is primarily determined by the dry-matter production per unit area — or rather per plant —, irrespective of the ecological conditions.

Higher dry-matter production involves a higher carbohydrate— and lower nitrogen concentration.

In plant production and plant breeding it is worth paying attention to this fact, since a higher dry-matter production means organic matter poorer in nitrogen.

When comparing the two varieties, we find that although the total carbohydrate content is higher in the variety "Kecskeméti konzerv" than in the "Kecskeméti törpe", there is hardly any difference in the nitrogen concentration between the two varieties. Table 5 summarizes the percentage values of energy utilization determined in the periods between transplantation and the first and second examination, respectively.

It can be established that energy utilization is higher in the "Kecskeméti konzerv" than in the "Kecskeméti törpe" (by about 60 per cent). This is mainly the result of the fact that the stand of "Kecskeméti konzerv" is denser and higher than that of "Kecskeméti törpe", further, temperature measured in the stand is lower in the case of "Kecskeméti konzerv." This is also proved by the correlation between the leaf-area index and energy utilization (Table 1).

Table 5

Energy conversion (%)

Time of transplantation	Kecskeméti konzerv		Kecskeméti törpe	
	1. exam.	2. exam.	1. exam.	2. exam.
First	3.2	1.3	2.6	0.3
Second	3.9	2.0	3.5	1.4
Third	5.3	7.8	4.6	3.9
Fourth	3.1	8.0	2.0	6.5

Maximum energy utilization was observed with a leaf area index of 0.6 in "Kecskeméti konzerv", and of 0.3 in "Kecskeméti törpe".

Energy utilization is the highest in both varieties 2—3 months following transplantation, later it decreases considerably. This phenomenon can be brought into connection with the age of the leaves and with the photosynthetic pigment system (SESTÁK—CATSKY 1962, GAFFRON 1960). Maximum energy utilization lasts for a longer period in "Kecskeméti konzerv" than in "Kecskeméti törpe".

Energy utilization on the average of the total vegetative period — with plants transplanted on the first and second occasion taken in consideration — was 1.5—3.0 per cent. Similar results were obtained by GAASTRA (1958) LOOMIS—WILLIAM (1963) and GIBBON *et al.* (1968).

Acknowledgements

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COMPARATIVE ANATOMICAL INVESTIGATIONS ON LOTUS CORNICULATUS AGG. III.

By

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Anatomical investigations on the roots of wild and cultivated taxons of *Lotus corniculatus* agg. reveal that the primary root is of triarch and tetrarch type, characteristical of the taxons. The secondary thickening begins about 6—8 weeks later. In the tissue structure of roots developed during the process of secondary thickening differences are shown at the small species and subspecies levels. So, as regards the different *Lotus* taxons, the secondary tissue differentiation of the root is of determinative character.

Introduction

During the anatomical treatment of *Lotus corniculatus* agg. after studies on the leaf and stem the tissue structure of the root — the subject of the present paper — was examined. As mentioned in earlier papers too (Borsos 1966, 1969, 1971). Numerous papers have been published on anatomical and embryological investigations into several genera of *Legumi- nosae (Papilionaceae)* ,first of all *Medicago, Vicia, Trifolium, Lupinus, Melilotus, Lathyrus.* etc. (Bugnon 1925, Goebel 1932, Simonds 1935, Soueges 1929, Watari 1934, Wilson 1913, Winton 1914, etc.), while as to the genus *Lotus* many hand-books only give references (Metcalf—Chalk 1957, Esau 1969). A morphological-anatomical treatment by H. W. Hansen (Hansen 1953) has so far been perhaps the only detailed relevant literary source based on research work. In this work Hansen briefly discusses — among others — the development of root tissues and the anatomical structure of older roots too. However, these descriptions only refer to the taxon called "*Lotus corniculatus* var. *vulgaris* Koch" in his experiments, that is, he makes generalizations from studies made on this taxon.

According to our own observations the tissue structure of the root — mainly its secondary thickening —, like that of the stem, is highly varied in the *Lotus* species, and characteristically different in the larger taxonomical units. This is, of course, in connection with the ecological differentiation of taxons determined by the habitat of growing.

Material and Methods

For the purpose of our experiment we chose *Lotus* taxons highly differing from one another, partly in morphological appearance, partly in the previously examined anatomical structure of leaf and stems. The test plants were grown from seeds collected at original habitats of the examined taxons, and in the case of cultivated types from seeds obtained from the Institute of Agrobotany, Tápiószele, and from the Agronomy Department of McGill University, Montreal — Ste. Anne de Bellevue. The examined taxons were:

Lotus corniculatus L. ssp. *corniculatus* var. *corniculatus*
Lotus corniculatus L. ssp. *hirsutus* (Koch) Rothm.
  var. *hirsutus* and
  var. *ciliatus*
Lotus tenuis W. et K.
Lotus borbásii Ujh.

Beside the wild taxons the following cultivated varieties of *Lotus corniculatus* were ncluded in the experiment: cv. "Wallace", cv. "Viking", cv. "Óvári" and cv. "Őrségi". The number of the examined plants was about 20.

Having been scarified the seeds were germinated in Petri dishes, on filter paper, at room temperature (about 22—24 °C). One or two days later germination started, and so the investigation began with the radicles of 2—3 days old seedlings. 6—7 days later the seedlings were planted in pots containing a mixture of soil and compost, and kept similarly at room temperature. Next time we made observations on the roots of one month old seedlings. The secondary tissue differentiation was examined on the roots of 2—6 months old plants transplanted into the experimental plots of the Botanical Garden of the Eötvös Loránd University. In addition, the anatomical properties of 2—3 years old highly lignified roots were studied on roots of taxons obtained from original habitats.

The large number of preparations (cross and longitudinal sections) were produced from the roots of young seedlings with the method of embedment (SÁRKÁNY-SZALAY 1964), and from older roots through excising by hand. The material of the latter was treated after Carnoy's fixation and Javel's alkalic clarification — with vezuvine-malachite-green double staining (SÁRKÁNY-SZALAY 1964).

Results

In the cited work of H. W. Hansen, in the chapter written on the development of the vegetative organs of *Lotus corniculatus* L. the histological development of the root and the structure of the tissues too are described. He points out that the root of *Lotus corniculatus* is of a triarch type. In the radicle of a few-day-old seedling an early differentiation of the stelar elements can be observed. Three protophloem initials and the threefold group of the protoxylem can be seen without the lignification of the walls of the tracheal elements. Between the xylem and phloem initials small arcs of parenchymatous cells are located. The outer layer of the stele is the single row of pericyclic cells. During the further development of the root the cell-wall thickening of protoxylem elements is apparent. The differentiation of the primary xylem is centripetal, and characteristic of a typical radial protostele. In the metaphloem sieve-tubes, companion cells and phloem parenchymatous cells are present. The endodermis has Caspary-stripes. Secondary growth was already observed by Hansen in the roots of 20-day-old seedlings. The arc of parenchymatous cells between the phloem and xylem groups continues to divide tangentially until a continuous cambium is formed. Its further activity produces the new xylem centripetally, and the phloem elements centrifugally. According to Hansen the triarch configuration becomes obscured in the older roots, and a complete vascular cylinder of xylem and phloem is formed. Beside the original parenchymatous medullary rays secondary ones too develop in the xylem and phloem rings. In the phloem the fibres appear in typical small groups. The first indications of lateral root initiation can be recognized in the pericycle. In older roots, the periderm is initiated by tangentional division in the pericycle. The phellogen produces layers of cork externally as well as a limited amount of parenchymatous phelloderm internally. Cork formation brings about the rupturing, disintegration, and eventual sloughing of the epidermis and primary cortex.

Hansen's above outlined anatomical characterization and observations of the root of *Lotus corniculatus* are highly valuable, but do not give a full picture at all. Our own investigations partly confirm Hansen's description, but — since we made a simultaneous comparison of more than one taxon — we add some supplements and characterizations which will complete the knowledge of the root anatomy of *Lotus corniculatus.*

1. The radicles of 2—3-day-old *Lotus corniculatus* seedlings studied by us are characterized by a small diameter central cylinder and a relatively developed primary cortex consisting of parenchymatous cells developing from the periblem. The central cylinder is filled in with the vascular bundles and some ground-tissue parenchyma. The star-like arrangement of the xylem components is already clearly seen at this early stage of development. It is of a triarch configuration in most taxons, while of a tetrarch appearance in *Lotus corniculatus* ssp. *hirsutus* (in two varieties) and cv. "Wallace". The tracheal elements have slightly thickened but not lignified cell-walls. Tracheae of wider lumen can be seen in the protoxylem of narrow lumen, 2—4 in the star-like arrangement, 5—6 in a branch in *Lotus tenuis.* The protophloem elements can be found between the star-shaped tracheal elements, separated by a one or two cell-row thick parenchymatous arc. The central cylinder is surrounded by the pericambium, a closely set cell row consisting of fully developed cells. It is joined outwards by cells of gradually increasing size, with undulate and rounded cell-walls, which form the primary cortex. The cells are loosely connected by smaller or larger, mainly triangular intercellular spaces. This parenchymatous ground tissue consists of 6—8 cell-rows in most taxons, and 4—6 in cv. "Óvári". The cross-section is completed by the outermost layer of the one-cell-row thick rhizodermis (Figs 1—4).

The radicle of a 2—3- day-oldseedling of *Lotus borbásii* is of peculiar appearance. Here too, the triarch arrangement of the stele elements is characteristic. The number of proto- and metaxylem elements in each point of the star is usually 4—5. The Caspari-striped endodermis is separated even in the initial phase of development. The primary cortex consists of 7—9 cell-rows, its

Cross- section of radicles from 2—3-day-old *Lotus* seedlings

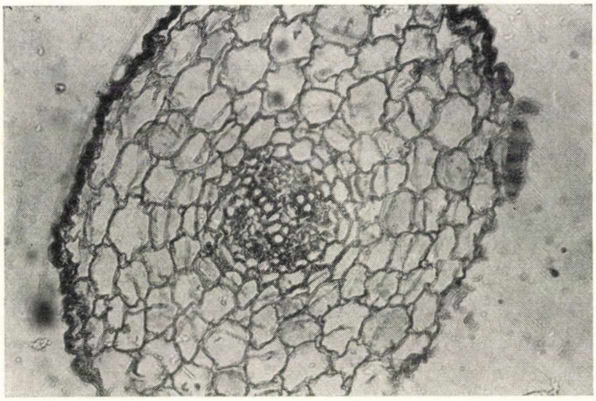

Fig. 1. cv. "Óvári" (12.5× ocul., 10× obj.)

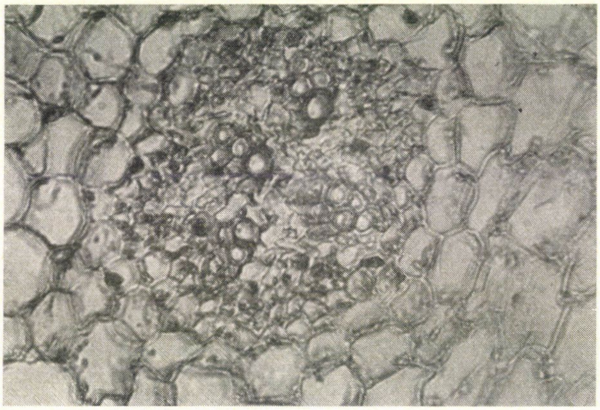

Fig. 2. L. corn. ssp. *hirsutus* var. *hirs.* (12.5× ocul., 25× obj.)

cells are smaller than in the other taxons, hexagonal or rounded, and closely set. Intercellulars are very few.

2. Tissue organization of one-month-old root. The outermost cell-row of the primary root, the rhizoderm, breaks up during the development, and is replaced by the exodermis which develops from the outermost cell-rows of the primary cortex. The exodermis generally consists of two cell-rows. The brick-shaped cells of the outer cell-row mostly fit together by undulate side-walls. The inner cell-row consists of larger, mostly square, radially slightly elongated cells with rounded corners (Fig. 7). The primary cortex underneath consists of large, rounded, thin-walled parenchymatous cells with smaller or larger intercellulars in between; it generally surrounds the central cylinder with 7—8 rows of cells. Its innermost cell-row adjacent to the peri-

Cross-section of radicles from 2—3-day-old *Lotus* seedlings

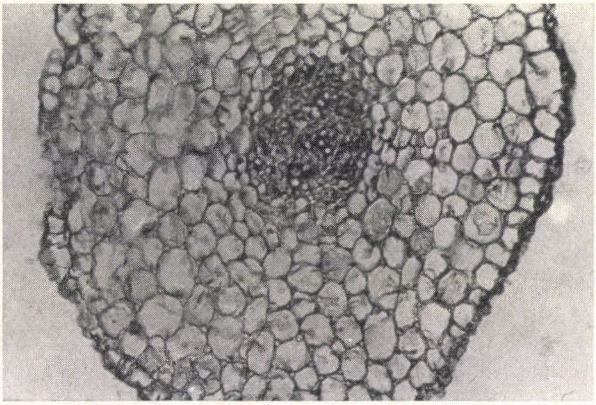

Fig. 3. L. corn. ssp. *hirsutus* var. *pilosus* (12.5× ocul., 10× obj.)

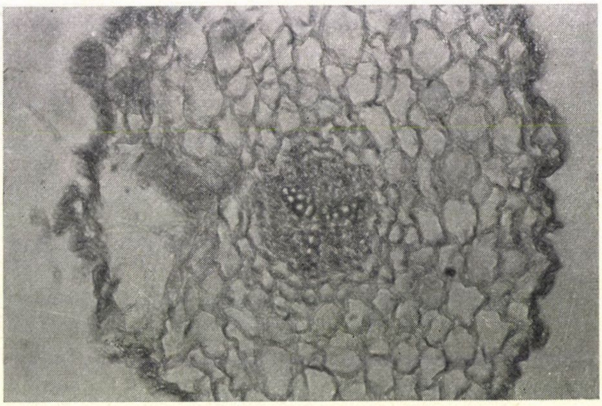

Fig. 4. L. tenuis (12.5× ocul., 10× obj.)

cambium is the Caspari-striped endodermis. In one-month-old *Lotus corniculatus* ssp. *hirsutus* and ssp. *corniculatus* the ground tissue cortex shows a very interesting development during which the walls of the parenchymatous cells are absorbed, larger cavities are produced through the fusion of two or more cells surrounding the central cylinder, first in a lower, while later in an increasing number (Fig. 8).

In the central cylinder the xylen bundles are arranged star-like, in triarch and tetrarch configurations. Beside the narrow-lumen protoxylem the wider lumen metaxylem elements appear with slightly thickened cell-walls, in larger numbers, in groups or cluster-like rows corresponding to the points of the star. The phloem bundles, easily distinguishable between the xylem rays, consist of sieve-tubes, companion cells and phloem parenchyma (Fig. 5). In most taxons the xylem bundles meet at the centre, so no central

Cross-section of roots of one-month-old *Lotus* seedlings

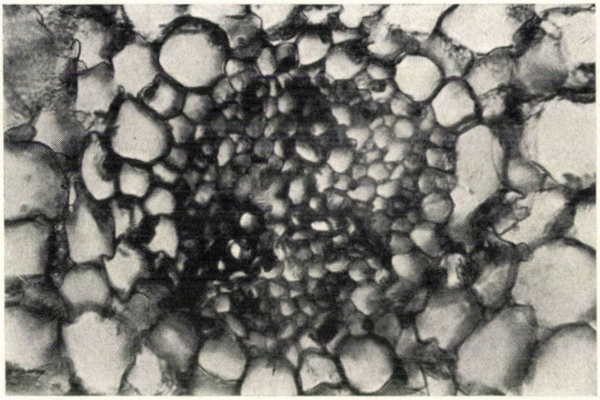

Fig. 5. L. corn. ssp. *hirsutus* var. *hirs.* (12.5× ocul., 63× obj.)

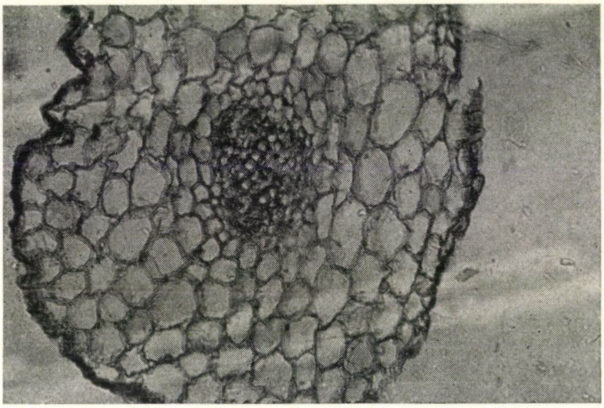

Fig. 6. L. corn. ssp. *hirsutus* var. *pilosus* (12.5× ocul., 10× obj.)

pith develops. The ground-tissue parenchyma is mostly represented by a single row of medullary ray between the xylem and phloem bundles (Fig. 6).

In the case of one-month-old seedlings the secondary thickening of the root cannot be pointed out yet, in contradiction to Hansen's description. Only the development of primary tissues can be spoken of here.

3. Secondary root organization of birds-foot trefoil. In the root of *Lotus* plants secondary thickening starts with the development of the undulate cambium. The latter develops into a connected cambium ring during the continuous tangential division of the parenchyma cells between the primary phloem and xylem groups, and with its bipleuric activity produces secondary xylem elements internally, and secondary phloem elements externally. The primary xylem bundles get inside the central cylinder; their triarch and tetrarch con-

Cross- section of one-month-old *Lotus* seedlings

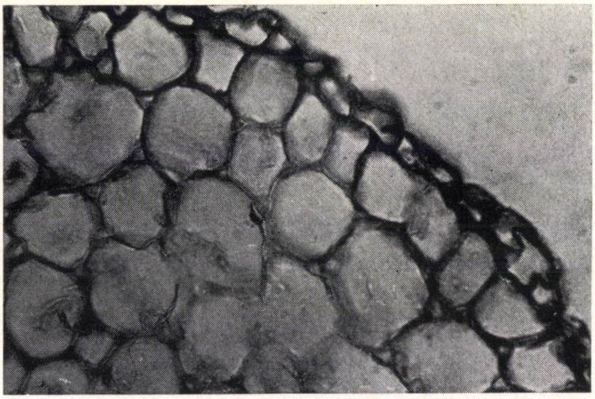

Fig. 7. L. corn. ssp. *hirsutus* var. *pilosus* (12.5× ocul., 63× obj.)

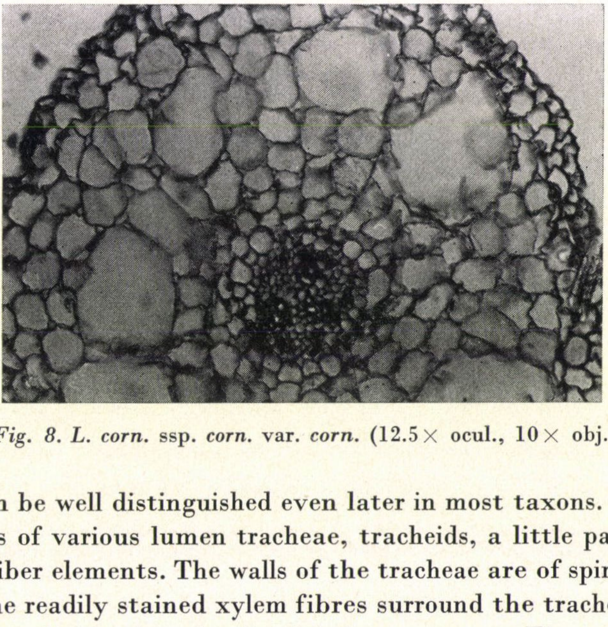

Fig. 8. L. corn. ssp. *corn.* var. *corn.* (12.5× ocul., 10× obj.)

figurations can be well distinguished even later in most taxons. The secondary xylem consists of various lumen tracheae, tracheids, a little parenchyma and many xylem fiber elements. The walls of the tracheae are of spiral and net-like thickening. The readily stained xylem fibres surround the tracheal elements in small groups, or divide them by forming larger groups. The secondary xylem elements develop into a radially arranged closed xylem cylinder which is divided partly by primary, partly by secondary medullary rays. The primary medullary rays are generally 2—3, while the secondary ones one or two cell-rows wide. The cells are, in all of them, radially elongated, narrow and thin-walled. In some taxons the primary medullarly rays broaden towards the centre. The pith is of rich starch content. Outwards from the several cell-row thick undulate cambium we find the secondary phloem which consists of sieve-tubes, phloem fibers and phloem parenchyma. The typical wedge-

Secondarily thickened roots of birdfoot trefoil

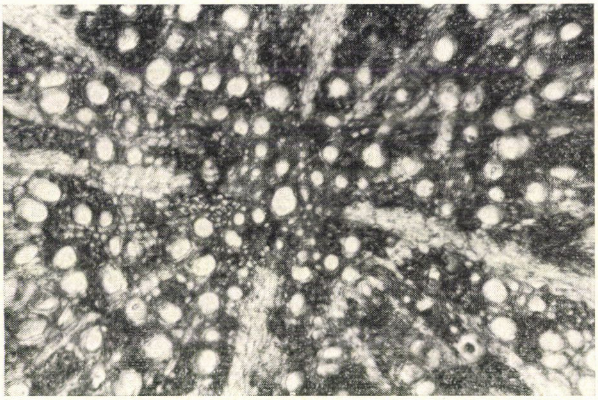

Fig. 9. cv. "Wallace" (cross-section, 12.5× ocul., 10× obj.)

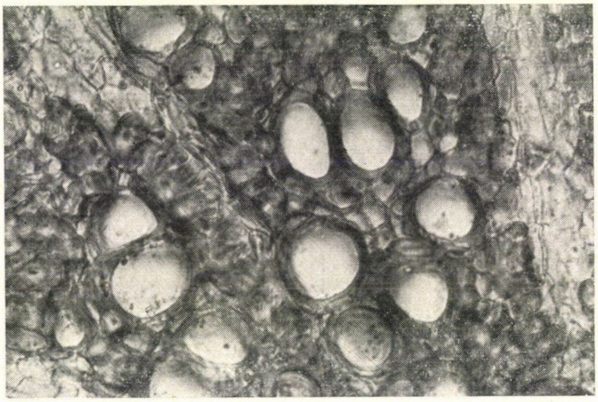

Fig. 10. cv. "Wallace" (cross-section, part of the xylem, 12.5× ocul., 63× obj.)

shaped pattern of the phloem is caused by the medullary rays crossing the xylem and continuing in the phloem. When reaching the phloem, they widen funnel-like through a dilatational growth by additional cell division. In the secondary phloem the smaller or larger groups of the phloem fibres are easy to stain. The elements of the primary phloem are difficult to distinguish as they are not sharply separated from the secondary phloem. Outwards the secondary phloem gradually passes over to the loose-structured, larger-celled parenchymatous tissue. The one-cell-row endodermis often becomes corky. The secondary phloem is surrounded from outside by the secondary bark produced by a dilatational growth outside the phloem; and the secondary bark is surrounded by the periderm. (Fig. 17). By a dipleuric activity the cork-cambium produces cork cells externally, and a phelloderm of ground

Secondarily thickened roots of birdfoot trefoil

Fig. 11. cv. "Wallace" (longitudinal section, calcium oxalate crystals in the phloem, 12.5 × ocul., 10 × obj.)

Fig. 12. L. corn. ssp. *corn.* var. *corn.* (part of the xylem, cross-section, 12.5 × ocul., 63 × obj.)

tissue character internally. In the phloem and the secondary bark a rich content of starch and calcium oxalate crystals is found.

4. As mentioned in the introduction, the secondary root thickening of wild taxons and cultivated varieties of *Lotus corniculatus* is diversified and of determinative character as regards the main taxons. Further on the secondary root thickening of characteristic taxons will be surveyed.

a) *Lotus corniculatus* L. ssp. *corniculatus* var. *corniculatus.* The primary xylem is of triarch configuration which remains distinct inside the root. In the secondary xylem the tracheae are very large, with wide lumen and thickened walls (Figs 12 and 25). The secondary xylem cylinder is broken up by the medullary rays. The primary medullary rays run radially at a width of 2—3 cell-rows to the centrally arranged primary xylem, while outwards widen funnel-like. The secondary medullarly rays are found at a width of one cell-row

Secondarily thickened *Lotus* roots

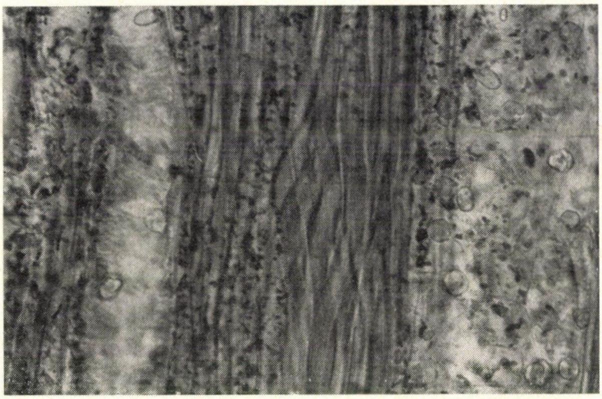

Fig. 13. L. corn. ssp. *corn.* var. *corn.* (tangential longitudinal section. 12.5 × ocul., 63 × obj.)

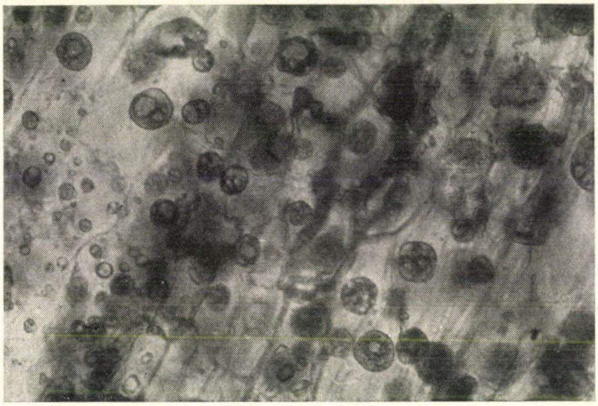

Fig. 14. L. corn. ssp. *corn.* var. *corn.* (tangential longitudinal section, starch grains; 12.5 × ocul., 63 × obj.)

in a shorter or longer decurrent, an irregular radial pattern between the xylem fibers and the tracheae. The secondary xylem cylinder is smaller compared to the phloem and bark. A rich content of starch is found in the parenchymatous tissue (Fig. 14).

b) cv. "Wallace".

The tetrarch configuration proto- and metaxylem is clearly seen in the centre of the root, though secondary xylem elements are also wedged in. The secondary xylem develops an unbroken cylinder in which the tracheae are of medium and large lumen, surrounded by smaller groups of tracheids and xylem fibers (Figs 9 and 10). It is characteristic that the cells of the xylem parenchyma divide the radial structure of the xylem by their concentric pattern. The primary medullary rays are 2—4-cell-rows wide, of radial arrange-

Secondarily thickened *Lotus* roots

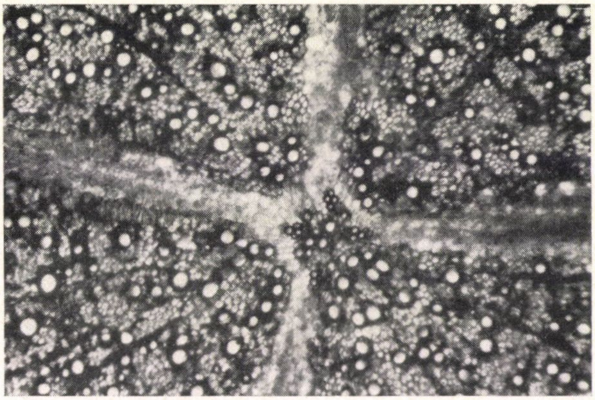

Fig. 15. L. corn. ssp. *hirsutus* var. *hirsutus* (cross-section, part of the xylem, 12.5× ocul., 10× obj.)

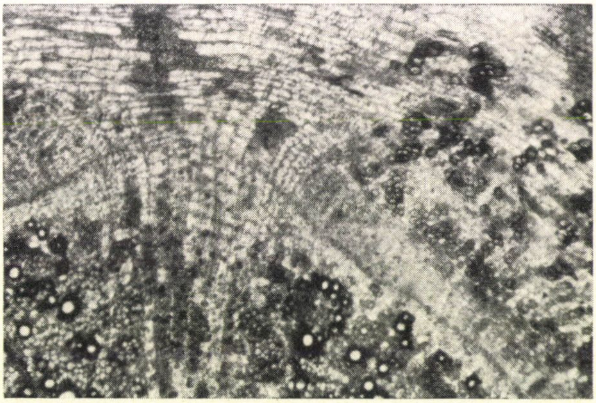

Fig. 16. L. corn. ssp. *hirsutus* var. *hirsutus* (cross-section, part of the xylem, 12.5× ocul 10× obj.)

ment, broadening about the middle. The secondary medullary rays too run radially, at a width of 1—2 cell rows. In the secondary phloem there is a large quantity of calcium oxalate crystals and relatively little starch (Fig. 11).

c) *Lotus corniculatus* L. ssp. *hirsutus* (Koch) Rothm.

The proto- and metaxylem inside the root are readily distinguished being surrounded by the parenchyma cells of medullary rays running to the centre (Fig. 15). The secondary xylem cylinder is well developed; the four primary medullary rays broaden about the middle, becoming 3—4-cell-rows wide, while in the phloem widen funnel-like (Fig. 16). The secondary medullary rays too run radially almost to the centre, and have a rich starch content. The tracheae are of narrow lumen, the xylem fibers well developed (Fig. 18). In the phloem there is a large number of readily stained phloem fibers. It is

Secondarily thickened *Lotus* roots

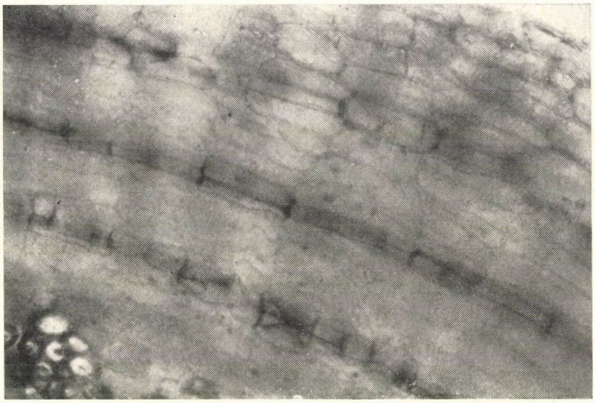

Fig. 17. L. corn. ssp. *hirsutus* var. *hirsutus* (cross-section detail: suberized endodermis, secondary bark, secondary epidermis; 12.5× ocul., 25× obj.)

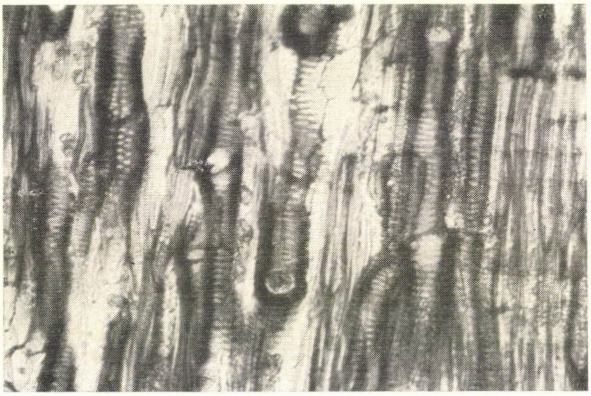

Fig. 18. L. corn. ssp. *hirsutus* var. *hirsutus* (radial longitudinal section, detail: tracheae, xylem fibers; 12.5× ocul., 63× obj.)

highly characteristic that the endodermis becomes corky, and the zone of the primary cortex is surrounded by the several cell-row wide secondary bark which is then closed by the periderm consisting of phelloderm and cork cells (Fig. 17).

d) *Lotus tenuis* W. et K.

It is characterized by a thick secondary xylem cylinder which is divided by a great number of (primary and secondary) medullary rays. The tracheae generally are of narrrow lumen, there are but a few with large lumen. A great many groups of xylem fiber develop (Figs 23, 24 and 25). The medullary rays are 3—5-cell-rows wide, and run to the centrally located primary xylem.

Secondarily thickened *Lotus* roots

Fig. 19. L. corn. ssp. *hirsutus* var. *hirsutus* (tangential longitudinal section, detail: calcium oxalate crystals; 12.5× ocul., 63× obj.)

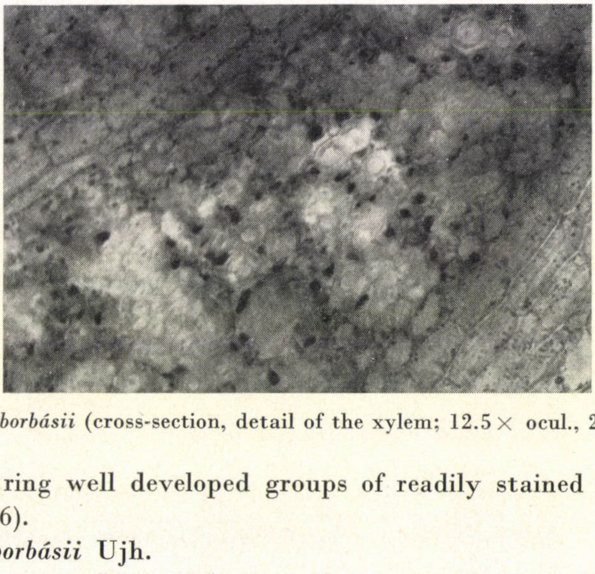

Fig. 20. L. borbásii (cross-section, detail of the xylem; 12.5× ocul., 25× obj.)

In the phloem ring well developed groups of readily stained phloem fibers are seen (Fig. 26).

e) ***Lotus borbásii*** **Ujh.**

During the secondary thickening of the root the secondary xylem becomes a well developed closed cylinder. A large number of narrow lumen tracheae arranged in radial rows accompanied by numerous xylem fiber groups are seen (Figs 20 and 22). In the inside of the root the triarch configuration of the primary xylem is surrounded by the broadening radial rows of medullary rays. In the phloem and the medullary rays starch and calcium oxalate crystals are found in large amounts (Fig. 21). The endoderm becomes corky, and outside the zone of the secondary cortex, as a result of the dipleuric activity of the cork-cambium, a thick phelloderm layer develops which is closed from outside by several rows of cork cells.

Secondarily thickened *Lotus* roots

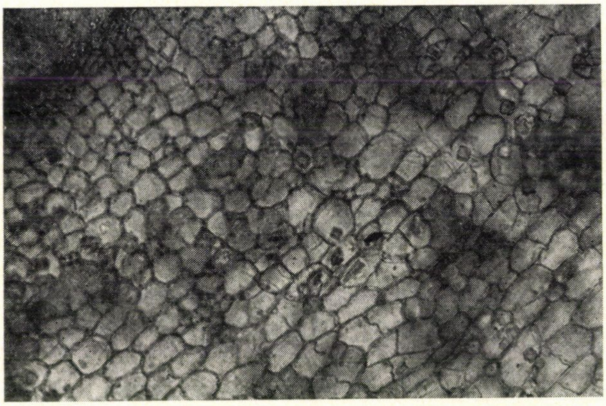

Fig. 21. L. borbásii (cross-section, detail: phloem with crystals; 12.5 × ocul., 25 × obj.)

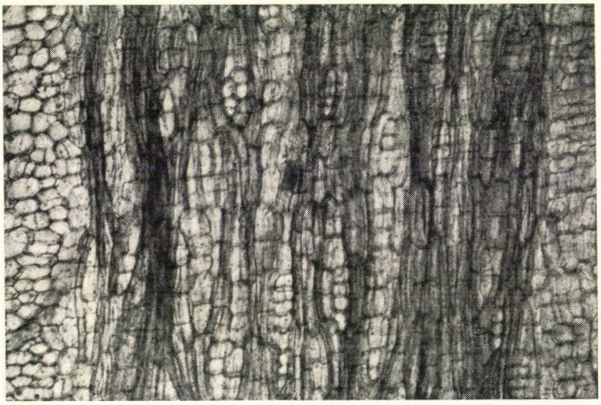

Fig. 22. L. borbásii (tangential long. section; 12.5 × ocul., 25 × obj.)

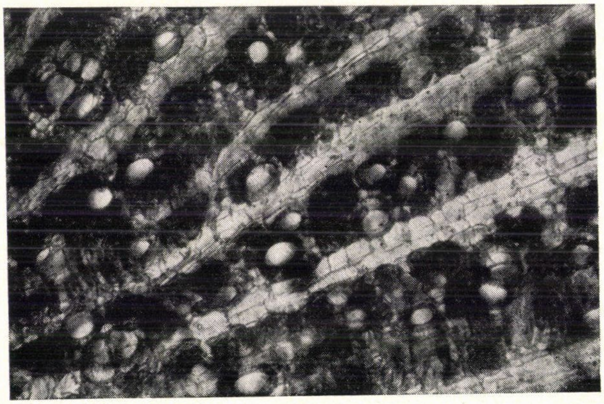

Fig. 23. L. tenuis (cross-section, detail of the xylem; 12.5 × ocul., 25 × obj.)

Secondarily thickened *Lotus* roots

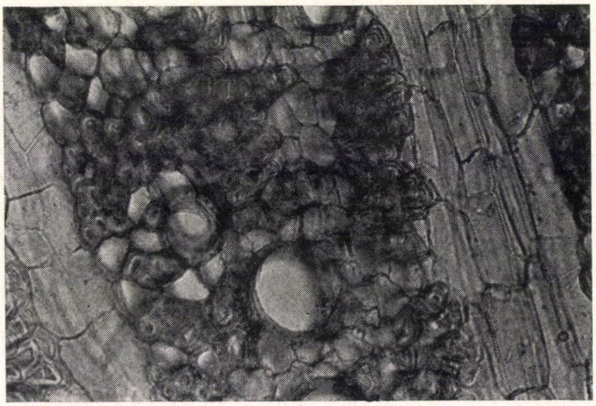

Fig. 24. L. tenuis (cross section, detail of the xylem; 12.5× ocul., 63× obj.)

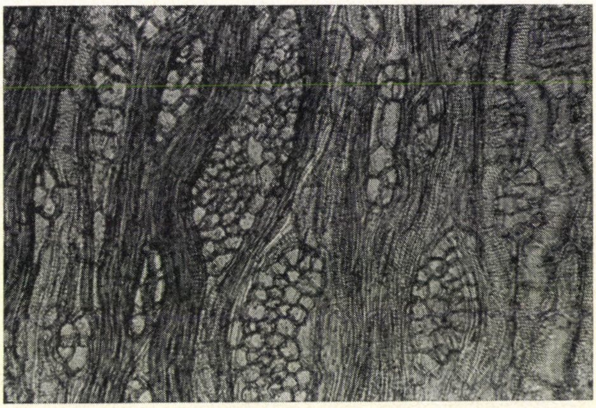

Fig. 25. L. tenuis (tangential longitudinal section, detail; 12.5× ocul., 25× obj.)

5. In the roots of several-years-old *Lotus corniculatus* taxons the same secondary thickening as outlined above is found, only, naturally, at a highly advanced stage of development. This is manifest mainly in a more intensive lignification, such as the thickening of the cell-walls of the tracheae, the well developed fibers and fiber groups, the extension of the suberized endodermis and phelloderm layers.

Acknowledgement

It is my pleasant duty to express my thanks to Mrs. Julia ZOTTER, laboratory assistant, for her help in the technical and calculation work.

Secondarily thickened *Lotus* roots

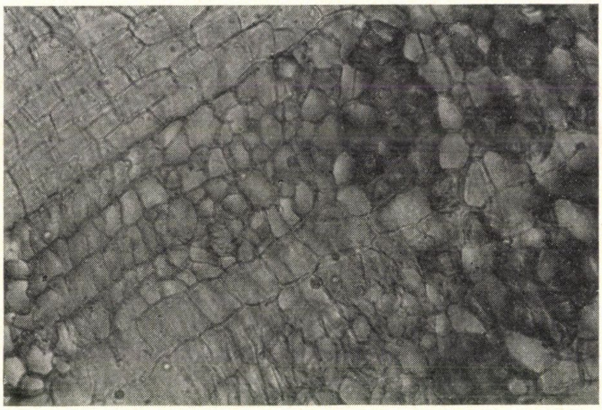

Fig. 26. L. tenuis (cross-section, part of the phloem; 12.5× ocul., 63× obj.)

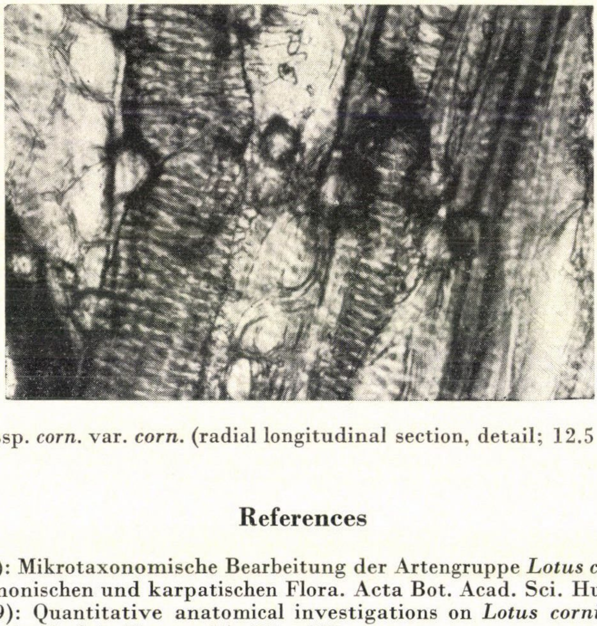

Fig. 27. L. corn. ssp. *corn.* var. *corn.* (radial longitudinal section, detail; 12.5× ocul., 63× obj.)

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POSSIBLE WAYS OF MORPHOGENESIS IN HIGHER PLANT TISSUE CULTURES*

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The aim of our work was to clear up what processes of cell and tissue differentiation take place in the callus tissue until the appearance of the plants (shoots, roots). According to our observations the development of the same organs may be preceded by totally different processes of differentiation. The same hormone concentration when acting in the callus tissues of different plant species, as well as different hormone concentrations in the callus tissue of the same species may induce either adventive embryogenesis or apical meristem differentiation. The paper deals with the adventive embryogenesis, the differentiation of the shoot and root apex as well as with the organogenesis. On the ground of research results obtained in the last several years we outline the possible ways of cell and tissue differentiation as well as of organogenesis enabling us to raise plants from undifferentiated callus cells.

Introduction

The correctness of Haberlandt's theory (1902) was confirmed by the scientific results of the past decades, since researchers succeeded in sustaining haploid and diploid cells and tissues of various plant species in vitro and regenerating plants from them thereby giving evidence of their totipotency in sterile cultures as well.

The results of morphogenesis induced in cell and tissue cultures have been summed up by a number of authors (Reinert 1962, 1968, Steward 1963, 1969, 1971, Halperin 1966, 1969, Gautheret 1966, Konar 1967—68, Wardlaw 1968, Wareing—Phillips 1970, Heszky 1971, 1973a) both in somatic and haploid cultures. In spite of this Maróti—Vágujfalvi—Domokos (1972), while carrying out investigations on this subject, arrived at the conclusion that "many data are available on the in vitro histogenetic and organogenetic differentiation of plants, but the mechanism and determination of differentiation have not been sufficiently clarified in spite of the large number of details known". In agreement with this statement we give an account here of our results concerning the mechanism of morphogenesis.

Some authors described an adventive embryogenesis (somatic embryogenesis, embryoid organization) in the differentiating somatic callus tissue, mostly in the callus tissue of carrot — an excellent test object of the last

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decade's study of somatic embryogenesis (REINERT 1959, HALPERIN—WETHERELL 1964, 1965, HALPERIN—JENSEN 1966, 1967, NAKAJIMA—YAMAGUCHI 1967, KATO 1968, SUSSEX—FREI 1968, REINERT—TAZAWA 1969, BACKS-HÜSEMANN—REINERT 1970). In vitro embryoid organization was observed by KONAR—NATARAJA (1965) in *Ranunculus sceleratus* species, by REINERT—BACKS—KROSING (1966) in *Umbellifera* culture species, by NORSTOG—RHAMSTINE (1967) in *Zamina integrifolia* and *Cycas circinalis* species and by RAO—NARAYANASWAMY—BEMJAMIN (1970) in *Tylophora indica.* Embryogenesis induced in callus culture corresponded to the development of an embryo formed from a zygote, as proved by HESZKY (1973b) at a several-cell stage of the proembryo as well.

From these results we can establish that somatic or adventive embryogenesis is one of the ways of morphogenesis, in the course of which the embryos developed on the culture medium differentiate, and by inducing the germination of embryoids we can raise plants from the callus.

The regeneration of plants in the callus tissue of various plant species has been reported by a number of authors — as e. g. VASIL—HILDEBRANDT (1966) for *Cichorium endivia*, TAKATORI—MURASHIGE—STILLMAN (1967, 1968) for *Asparagus officinalis*, LUSTINEC—HORÁK (1970) and HORÁK—LANDA—LUSTINEC (1971) for *Brassica oleracea*, ZAGORSKA—SAMINA—BUTENKO (1971) for *Nicotiana tabacum*, NISHI—YAMADA—TAKAHASHI (1968) for *Orysa sativa*, RAO—NARAYANASWAMI (1968) for *Solanum xanthocarpum* and HEINZ—MEE (1969) for *Saccharum* species — but in some cases the process and induction of differentiation differed from the adventive embryogenesis. The results proved, at the same time, that the morphogenesis may take courses different from those of the adventive embryogenesis. In the course of cytological and histological studies shoot and root organization was observed in several cases.

Shoot bud organization was reported by KONSTANINOVA—AKSENOVA—BAVRINA—CHAYLAHYAN (1969) and KOCHHAR—BHALLA—SABHARWAL (1971) in *Nicotiana tabacum* and by HILL (1968) in *Chrysanthenum* cultures. On the other hand, in the callus tissues of potato (OKAZAWA—KATSURA—TAGAWA 1967), wheat (TRIONE—JONES—METZGER 1968, DUDITS 1972) and haploid rice (HESZKY—SAJÓ 1973) only root apex differentiation and rhizogenesis were generally observed even under the influence of various hormone concentrations and interrelations.

In a classical experiment SKOOG—MILLER (1957) could induce either bud formation or root formation in tobacco callus tissue culture by changing the hormone concentrations and interrelations. Their results were only moderately confirmed by experiments repeated with various plant species. In some species shoot, in others root formation was easier to induce. In many cases the other pole had to be regenerated on organs already developed. The probability of shoot formation and development was more frequent in callus cultures of *Germanium* varieties (Pillai—Hildebrandt 1969), haploid *Datura metel* (Iyer—Raina 1972), *Trifolium repens* (Pelletier—Pelletier 1971) and haploid tobacco (Tanaka—Nakata 1968). From the callus tissues of monocotyledons root development was generally found easier to induce.

As seen from the results the questions of morphogenesis are not perfectly clear; some authors describe the processes of differentiation and organogenesis differently. It can be established, however, (Fig. 1) that morphogenesis can be induced in the callus tissue cultures of higher plants in different ways

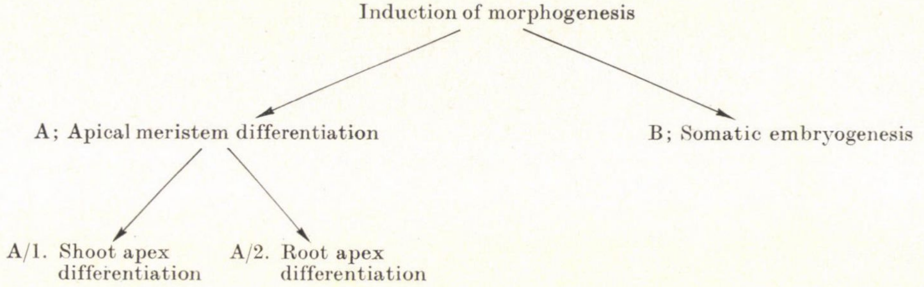

Fig. 1. Possible ways of inducing morphogenesis in the callus tissue cultures of higher plants

(Heszky 1972). The afore given results can all be placed in one or another of the two or the three types seen in Fig. 1. It is supposed that all the three forms of morphogenesis can be induced in the callus tissue of each plant species. This has, however, indispensable preconditions, namely, an adequate composition culture medium, and the optimum quantity and ratio of hormones established by experiments.

Material and Method

Carrot callus was induced from the root tissue of the variety "Weszraja vennaja". Sterile fragments — including the cambial zone — of one year old roots were placed on RM medium (Linsmayer—Skoog 1965) completed by 2.4 D (2.0 ppm) and IAA (1.0 ppm) or NAA (4.0 ppm). Proliferation started from the cambial zone and the paranchyma tissue surrounding it, 2—3 weeks after the isolation. The developing callus was swelled through several passages on a RM culture medium supplemented with IAA (4.0 ppm), kinetin (0.08 ppm) and 2.4 D (4.0 ppm). The undifferentiated callus tissue thus increased was used for studying the morphogenesis.

The tobacco callus tissue was produced by inducing callus formation in sterile haploid plantlets. From anthers of the variety "Szabolcsi" haploid plants were raised with the help of the method and culture media described by Nitsch—Nitsch (1969) (Heszky—Paál 1972). The haploid plantlets were placed on RM culture medium supplemented with IAA (4.0 ppm), kinetin (0.08 ppm) and 2,4 D (4.0 ppm). The developing callus was swelled through repeated passages and used to investigate the morphogenesis.

With the purpose of inducing and maintaining the morphogenesis and raising plants the undifferentiated callus tissues were placed on *RM* culture media containing IAA (2.0—8.0 ppm), kinetin (0.02—1.0 ppm) and NAA (1.0 ppm). The tissue cultures were kept in natural light completed by artifical illumination (16 hours/day), at a temperature of 25—28 °C.

In the callus preparations stained with carmine acetic acid we studied the phases of cell, tissue and organ differentiation. From the differentiating callus tissues sterile samples were taken every 1—3 days. The photos were made with a MOM tube (0.6—40 × objective, 2.5—6.3 × projective) fitted to a "Laboval" Zeiss microscope or Zeiss stereomicroscope, on NP 20 film.

The chromosome number was determined in preparations made from the root tips of plantlets grown in Knop solution by the generally used carmine acetic acid method.

When only a single organ developed from the callus we used *RM* culture medium supplemented with NAA (1.0 ppm), Difco Orchid culture medium (HESZKY 1971) or Knop solution to release the growth inhibition, or regenerate the opposite pole.

Results

I. Investigation on adventive embryogenesis in the somatic callus tissue culture of Daucus carota L.

The undifferentiated callus tissue was placed on a culture medium containing 2.0 ppm IAA and 0.02 ppm kinetin with the purpose of inducing embryogenesis. Beyond the aim of inducing embryogenesis we wanted to repeat the interrelation tests and results of SKOOG—MILLER (1957) in the callus tissue of the carrot.

Four to six weeks after the isolation tiny plantlets — or in the case of 0.02 ppm kinetin added to 2.0 ppm IAA roots, and with a kinetin concentration of 1.0 ppm shoots — appeared in the callus tissue. So we succeeded in repeating Skoog — Miller's interrelation experiments in carrot callus tissue too. However, continuous cytological investigations made on the differentiating callus revealed that prior to the development of either shoots or roots embryoids were formed in the callus, and the interrelation dependent difference could not be pointed out in the adventive embryogenesis. The difference only occurred with the germination of the embryos, caused by the growth inhibition of either the root or the shoot apex of the embryo.

The cytological investigations enabled us to follow the full embryogenesis of embryoids developing from a single cell, with special regard to the several-celled proembryo stage — not perfectly clarified in the literature — described in detail in an earlier paper (HESZKY 1973b). In the present paper it is only referred to in Fig. 2. According to the investigations the organization of embryoids developing from callus cells becoming embryonal agreed with the development of the embryo taking shape from the zygote both in a several-celled state and at the subsequent stages.

Fig. 3/A shows torpedo-stage embryoids differentiating 3—4 weeks after the isolation through the elongation of the radicle and hypocotyl primordia. After this the development of the cotyledon stopped — as in the in vitro embryo cultures —, but in the mature embryoids developed after a further organization the primordia of procambial budle sheat fundaments could already be clearly distinguished (Fig. 3/B).

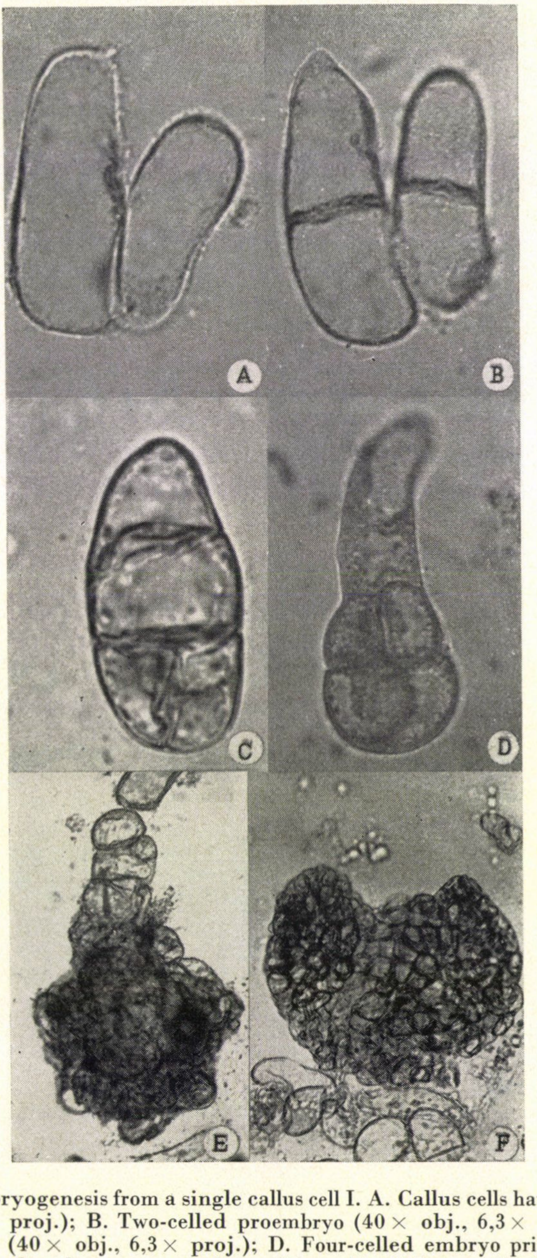

Fig. 2. Somatic embryogenesis from a single callus cell I. A. Callus cells having become embryonal (40 × obj., 6,3 × proj.); B. Two-celled proembryo (40 × obj., 6,3 × proj); C. Four-celled T-shaped proembryo (40 × obj., 6,3 × proj.); D. Four-celled embryo primordium (40 × obj., 6,3 × proj.); E. Globular-shape stage embryo (40 × obj., 2,5 × proj.); F. Heart-shape stage embryo (10 × obj., 4,0 × proj.)

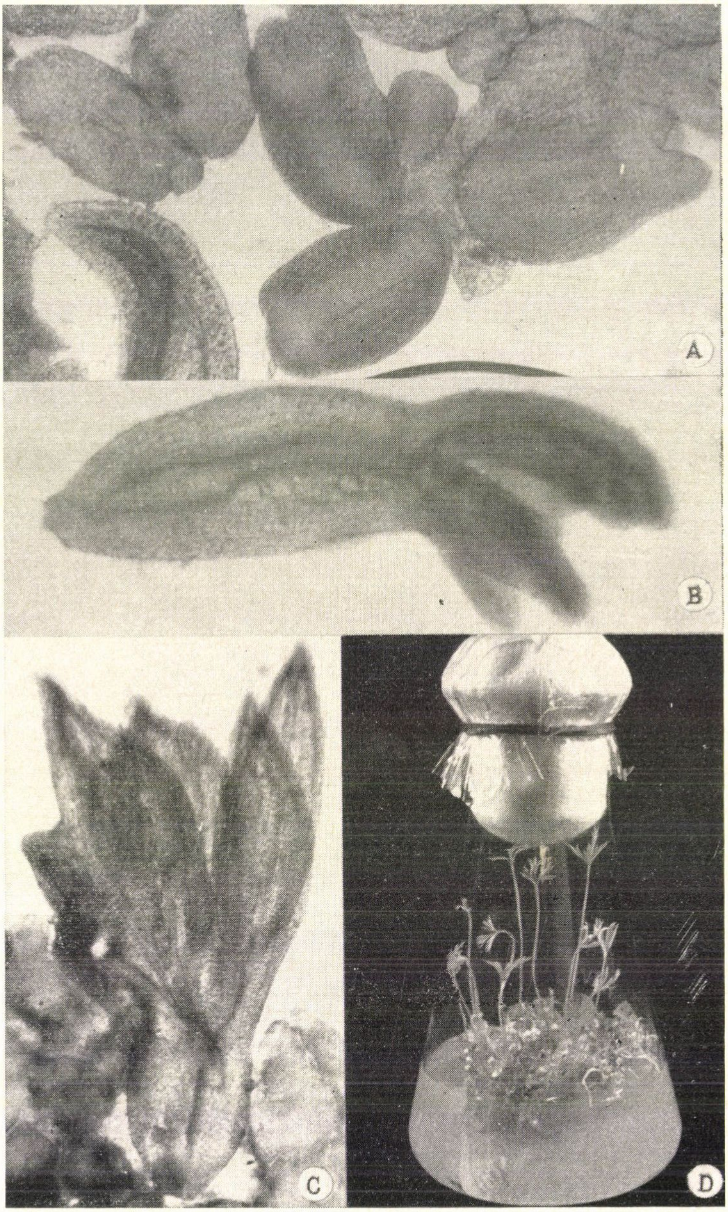

Fig. 3. Somatic embryogenesis from a single callus cell II. A. Torpedo-stage embryo (10 × obj., 2,5 × proj.); B. Fully developed embryo (10 × obj., 2,5 × proj.), C. Shoot formation of an embryo with the growth inhibition of the root apex (10 × obj., 2,5 × proj.); D. Shoots raised from callus

No difference was found in the embryogenesis as a response to changing kinetin concentrations compared to a constant 2 ppm IAA level. The embryogenesis was normal on both culture media, difference was only shown in the organogenesis.

From the fifth to sixth week after isolation the development of the embryoids became confused. In the case when the culture medium — where the embryogenesis was induced — contained 2.0 ppm IAA and 1.0 ppm kinetin after the embryogenesis, only the initial cells of the shoot apex and the tissues of the primary meristem showed activity, and from the callus tissue only the organogenesis of the shoot could be observed (Fig. 3/c). Even later only shoot formation could be seen from the callus (Fig. 3/D).

Fig, 4. Somatic embryogenesis from a single callus cell III. Root formation of an embryo with the growth inhibition of the shoot apex (4× obj., 2,5 × proj.)

When the culture medium was supplemented with 2.0 ppm IAA and 0.02 ppm kinetin, similar disorders occurred in the development of the embryo after it had reached the torpedo stage. Beside a normal growth of the initial cells and meristemic tissues of the root the growth of the shoot apex was inhibited (Fig. 4). Even in the later periods of the culture only root formation could be observed from the callus tissue.

The plantlets possessing roots or shoots were then placed on a simple Difco-Orchid culture medium (Fig. 5/A), or in Knop solution (Fig. 5/B), where the development of the earlier inhibited organs was normal. Seedlings possessing roots and shoots were transplanted into soil (Fig. 5/C). The further growth of the plants did not differ from that of plants raised from seed (Fig. 5/D), and their chromosome number was 2n = 18.

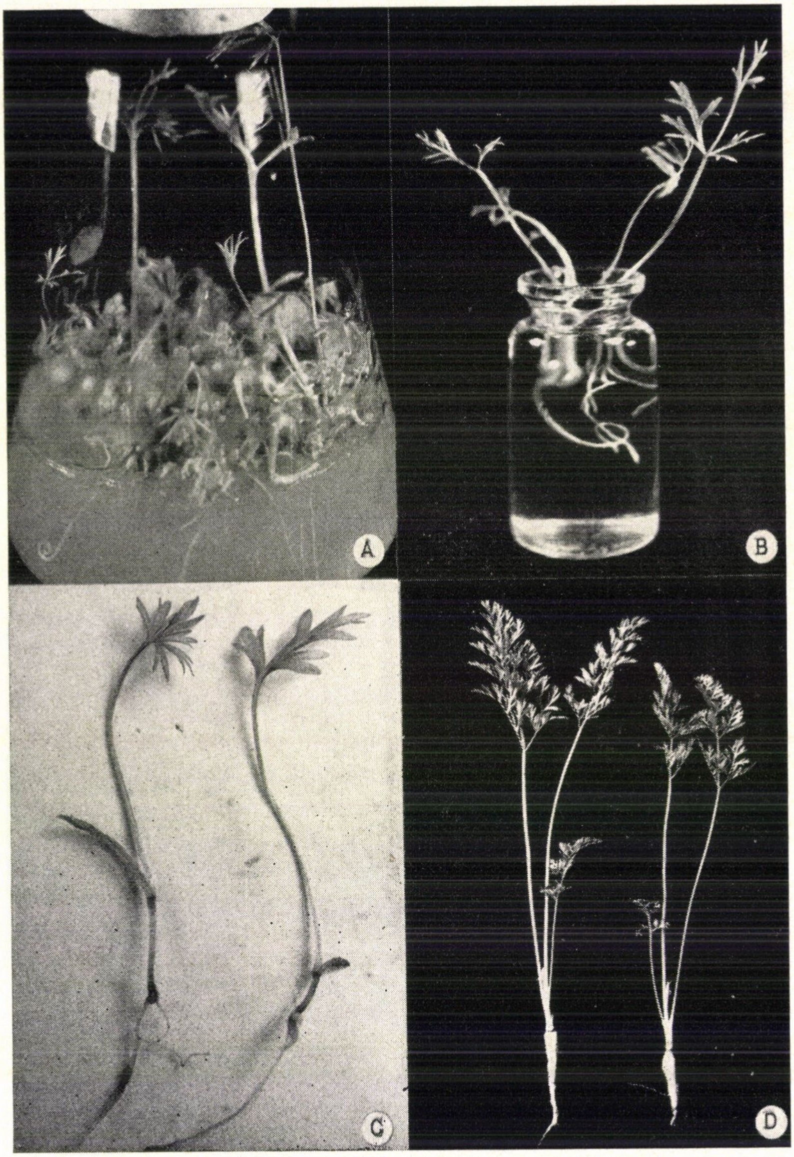

Fig. 5. Plants raised from embryos having differentiated on culture medium. A. Root formation induced on soil Difco-Orchid culture medium; B. Root formation induced in Knop solution; C. Plantlets with roots and shoots; D. Fully developed plants

II. *Investigation on apical meristem differentiation in a haploid callus tissue culture of Nicotiana tabacum L.*

Shoot and root apice differentiation was induced on *RM* culture medium supplemented with IAA (2.0 ppm) and kinetin (0.02—1.0 ppm), according to the hormone interrelation tests of Skoog—Miller (1957). On the culture medium 3—6 weeks after the isolation — depending on the supplementation — either shoots or roots developed from the callus.

Examinations of cytological preparations made from the callus tissue before the appearance of the different organs revealed that the histo- and organogenetic processes observed in the tobacco callus tissue were different from the mode of differentiation of the carrot callus tissue — somatic embryogenesis — in spite of the identical culture media used in the two cases.

Following the isolation from the undifferentiated callus cells (Fig. 6/A) tracheid cells differentiated with net-like cell-wall thickening (Fig. 6/B). Callus cells having differentiated into one-celled tracheids located in the callus tissue in thread-like or spherical groups (Fig. 6/D). In the latter case tiny round cells with large nuclei differentiated around the tracheid centres (Fig. 5/C). The frequent division of these cells resulted in the development of a promeristem. This stage suggested the differentiation of the apical meristem in the callus (Fig. 6/D).

With the further organization of the apical meristem the protoderma — a one-cell-row surface meristem differentiating from the outer cell layer of the promeristem — can be clearly distinguished, and as a result of a further differentiation trichomes appear on it (Fig. 6/E). During the continued organization of the shoot apex the differentiating leaf primordia can be seen in the form of protuberances (Figs. 6/F, 7/A). Subsequently, with the differentiation of the corpus a shoot apex characteristic of the tobacco plant develops slowly (Fig. 7/C); the figure clearly shows that in the case when the shoot apex differentiates the root pole does not develop.

The differentiation of the root apex was characterized by a meristem formation similar to that described above.

Fig. 7/E shows a root developing on *RM* culture medium supplemented with IAA (2.0 ppm) and kinetin (0.02 ppm). Around a tracheid centre more than one meristem tip may develop as illustrated in Fig. 7/D for roots and Fig. 7/B for shoots.

The large number of apices do not start developing in every case. There are cases when neither shoots nor roots develop from the apices formed. This can be explained by the fact that in certain cases the composition and osmotic pressure of the culture media required for the differentiation of the apical meristem are different from what is necessary to induce and maintain the development of shoots and roots from the apices.

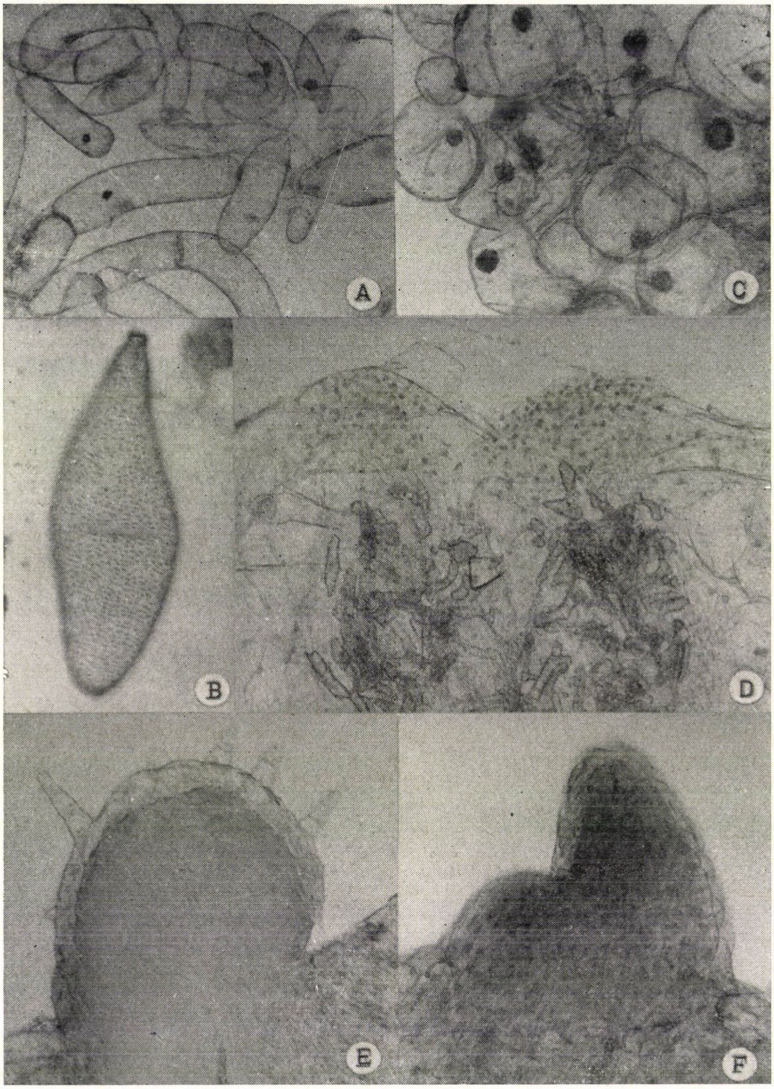

Fig. 6. Apical meristem differentiation from callus cells I. A. Undifferentiated callus cells (40 × obj., 6,3 × proj.); B. One-celled tracheid differentiated from a callus cell (40 × obj., 6,3 × proj.); C. Meristem cells differentiated from callus cells (40 × obj., 6,3 × proj.); D. Promeristem tissue differentiating around a tracheid centre (10 × obj., 6,3 × proj.); E. Epidermis having differentiated from the protoderm of the shoot apex, with trichomes (10 × obj., 6,3 × proj.); F. Protuberance indicating the differentiation of a leaf primordium (10 × obj., 6,3 × proj.)

Cultures containing developing shoots (Fig. 8/A) were placed on culture medium supplemented with NAA (1.0 ppm) where the roots regenerated in a few weeks (Fig. 8/B). Plantlets possessing shoots and roots were placed first in Knop solution (8/C) then planted into soil (8/D). The chromosome number of the raised plantlets was varying (2n = 24, 36, 48, 72).

Discussion

Undifferentiated callus tissues of carrot and tobacco were placed on culture media of identical composition at the same time. When 2.0 ppm IAA and 0.02 ppm kinetin was added to the culture media roots, whereas when 2.0 ppm IAA and 0,08 ppm kinetin was supplemented shoots developed from the undiferentiated callus tissues, in accordance with the hormone interrelations. Thus we succeeded in repeating the interrelation tests of SKOOG—MILLER (1957) with haploid tobacco callus- and somatic carrot callus tissues. However, on the grounds of cytological investigations it could be established that the appearance of the same organs was preceded by different processes of differentiation in the callus tissues of the two species. The same culture medium induced adventive embryogenesis in the carrot, and apical meristem differentiation in the tobacco callus.

As seen in Fig. 9 in the somatic callus tissue of the carrot some callus cells became embryonal following the induction of morphogenesis, divided in the same way as a zygote, and embryoids differentiated from them (Fig. 9, 1—12). However, the embryogenesis is not always normal. When an improper culture medium is used induction may take place and embryogenesis start, but the embryos will become degenerated, callous, etc. at a definite stage of development. It is not sure either that plantlets will develop from the embryoids even if the full embryogenesis is successfully maintained. It may occur that the callus containing the fully developed embryos has to be placed on a new culture medium to release the growth inhibition of the different organs and induce germination. It is, however, a much more frequent case that on the culture medium where the embryogenesis has been induced germination starts as well. If everything turns out well both root and shoot formation are normal, in this case plantlets possessing shoots and roots will develop on the culture medium (Figs 9, 13—15). But in other cases either just the root or just the shoot develops (Figs 9, 16—17 and 21—22). In such cases the developing embryos have to be placed on a new culture medium where the growth inhibition of the opposite pole will be released. The Difco-Orchid culture medium or the simple Knop solution are excellently suitable for this purpose. On these culture media the opposite poles begin to develop whereby plants possessing roots and shoots will be obtained (Figs 9, 18—20 and 23—25).

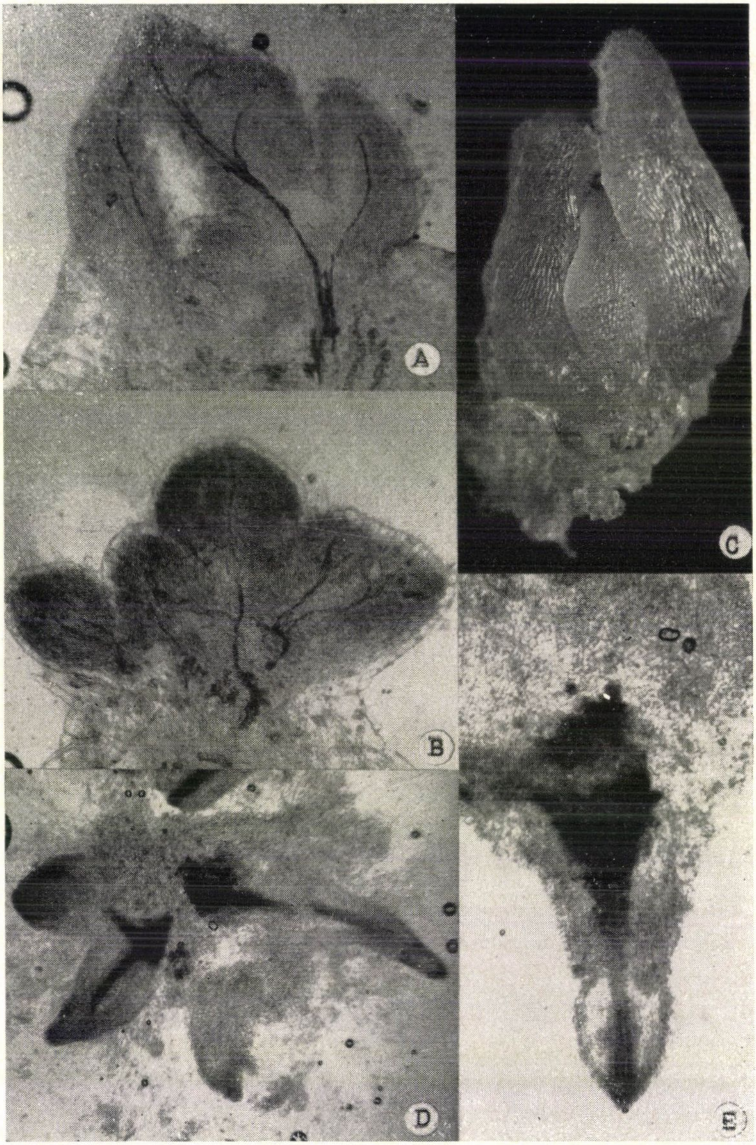

Fig. 7. Apical meristem differentiation from callus cells II. A. Differentiating shoot apex and leaf primordium (10 × obj., 4 × proj.);B. Shoot apices differentiating around a tracheid centre (10 × obj., 2,5 × proj.); C. Shoot apex differentiated in callus tissue (2 × obj., 2,5 × proj.); D. Root apices differentiating around a tracheid centre (10 × obj., 2,5 × proj.), E. Root differentiation from callus tissue (10 × obj., 4 × proj.)

Fig. 8. Raising plants from apices differentiating on culture medium. A. Numerous shoots developing from the callus after the differentiation of the shoot apex; B. Root regeneration on culture medium containing 1 ppm NAA; C. Root regeneration in Knop solution; D. Tobacco plants raised from callus tissue (from the left to the right: 2n = 48 and 2n = 36)

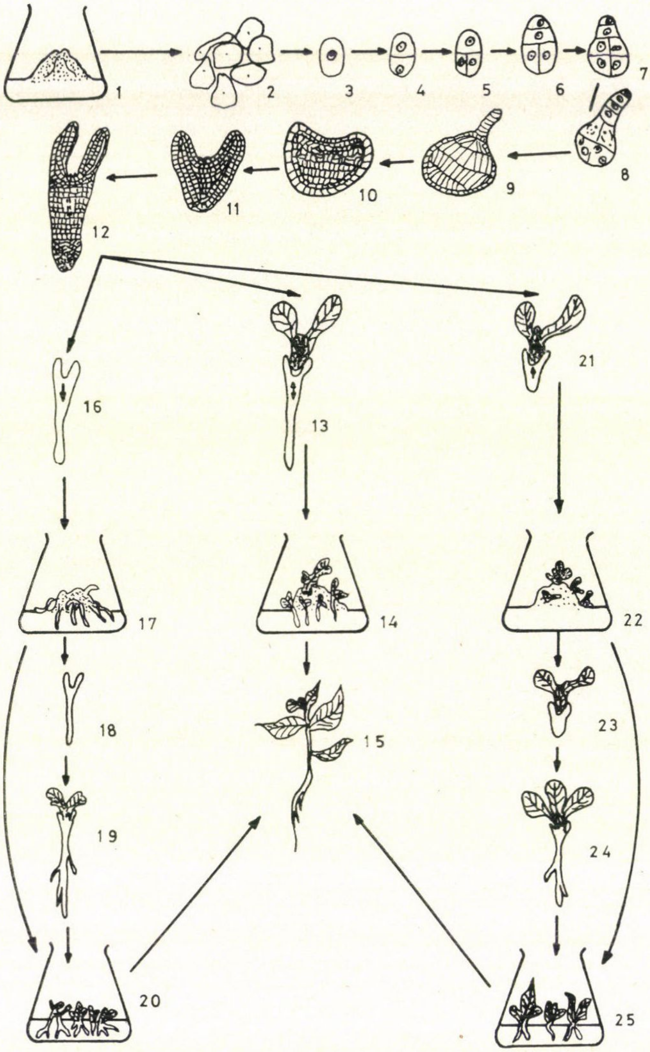

Fig. 9. Possible ways of morphogenesis in the callus tissue of higher plants I. Somatic embryogenesis. 1. Undifferentiated callus tissue; 2. Callus cells; 3. Callus cell having become embryonal, 4—12. Adventive embryogenesis; 13. Shoot and root formation of embryo; 14. Shoot and root development from callus; 15. Fully developed plant; 16. Root formation of embryo; 17. Roots developing from callus; 18—19—20. Induction of shoot development; 21. Shoot formation of embryo; 22. Shoots developing from callus; 23—24—25. Induction of root development

In Fig. 10 in the haploid callus tissue of the tobacco plant following the induction of morphogenesis a number of callus cells simultaneously differentiate into one-celled tracheid cells by a net-like cell-wall thickening. Around the tracheid cells located in groups tiny round cells with large nuclei differentiate, which by repeated cell division produce the promeristem (Figs 10, 1—4). During the continued organization of this meristem either a shoot or a root apical meristem differentiates in the callus (Figs 10, 5—7, and 11—13). The culture medium inducing and maintaining the apice differentiation is generally suitable for promoting the further development of shoot apices and root apices, so either mere shoots (Figs 10, 8) or mere roots (Figs 10, 14) develop from the callus. If we are to raise plants the opposite pole has to be regenerated which is easier in the case of the root. The Difco-Orchid culture medium or the Knop solution are also suitable for this purpose (Figs. 10, 9—10), but shoot regeneration generally necessitates experimentation with new culture media (Figs 10, 10—15).

Conclusions

In higher plants tissue culture morphogenesis has two forms: somatic embryogenesis and apical meristem differentiation (Figs 11). It is important to know about this, since it may happen in both cases that from the callus either mere roots (Figs 9, 17, and 10, 14) or mere shoots (Figs 9, 22, and 10, 8) develop, but the organs are the results of perfectly different processes of differentiation.

Somatic embryogenesis is the result of the organization of a callus cell, from which we can conclude that fully developed plants can only be reproduced from cell cultures through the induction of somatic embryogenesis.

Apical meristem differentiation is the result of the simultaneous organization of numerous callus cells, so this form can only be induced in tissues. That is the reason why plants could be raised from the callus tissue in much more plant species, than from the cell cultures.

Apex differentiation can be regarded as a simpler way of morphogenesis, it is easier to induce than somatic embryogenesis in the callus tissue of most plant species. That is the reason why those dealing with plant tissue cultures are more successful in proving the totipotency than those occupied with animal and human tissue cultures, where in the future fully developed organisms can only be reproduced by inducing somatic embryogenesis. In each plant species morphogenesis can be induced in both ways, only the proper culture medium has to be experimentally determined in each case, and this is often a lengthy procedure.

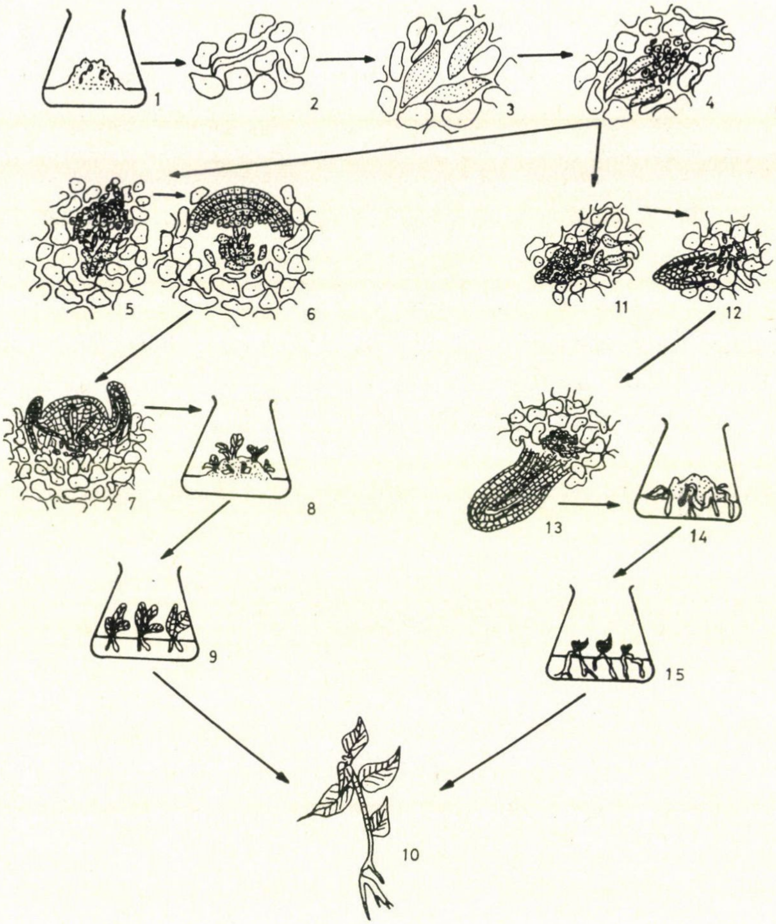

Fig. 10. Possible ways of morphogenesis in the callus tissues of higher plants II. Apical meristem differentiation. 1. Undifferentiated callus tissue 2. Callus cells; 3. Differentiation of callus cells into tracheid cells; 4. Differentiation of meristem cells around a tracheid centre; 5—6—7. Differentiation of shoot apex; 8. Shoots developing from the callus; 9. Induction of root regeneration on a new culture medium; 10. Fully developed plant; 11—12—13. Differentiation of root apex; 14. Roots developing from the callus; 15. Induction of shoot regeneration on new culture medium

Testing in callus culture the biological activity and interrelation of various subtances are many cases confined to the appearence of the organs (roots, shoots), or their number. Conclusions thus obtained may be completed, or disproved by the results of cytological investigations on the processes of cell and tissue differentiation taking place in the callus tissue and preceding the appearance of the organs, since often even in those cases when neither root nor shoot develops from the callus the induction of morphogenesis can be observed, only the differentiation stops or becomes confused at a certain point.

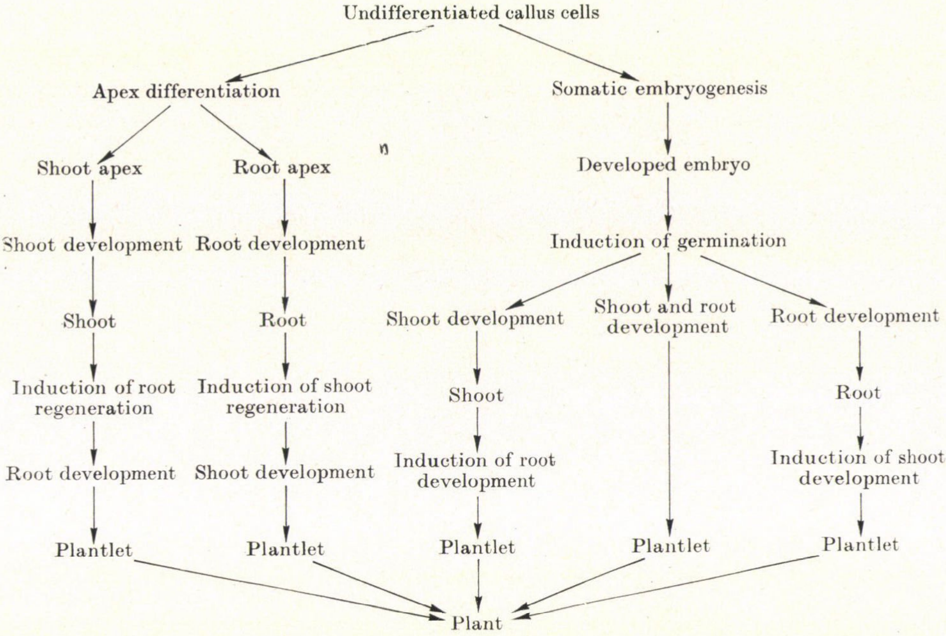

Fig. 11. Possible ways of raising plants from the callus tissues of higher plants

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VARIA

RED PEPPER KALOCSAI

Determinate 601

Taxonomical place: *Capsicum annuum* L. convar. *longum* (DC) Terpó provar. *rectum* Fingerh. conc. *kalocsaiense* My. subconc. *erectum* My. (TERPÓ 1965).

Origin: an early progeny obtained from Kalocsai 57–231 × *Capsicum annuum* L. "var. *fasciculatum*" by selection and back-crossing (TUZA–HORVÁTH 1973).

Breeders: Ferenc Márkus and Károly Kapeller, Kalocsa.

State qualification: provisionally certified variety, 1971.

General characterization: a determinate, rosette-type, high pigment content, non-hot red pepper variety with erect fruits ripening 10–14 days earlier than the parent form.

Morphological description:

Root system: medium strong, penetrating to 50 cm at the most.

Shoot system: 30–35 cm high, simply developed and of determinate growth habit, with a cymose branch system of 3–4.

Stem: on the 20—30 cm long main axis the internodes are 2—5 cm long, of yellowish green colour; short cymose branch systems at the apex.

Foliage: thin, with leaves mostly developing below the fruits. The leaf blades are broad, lanceolate, leathery, of dark yellowish green colour with long petioles. After flowering new leaves no longer develop.

Flowers: The corolla is whitish yellow; the flowers blossom at the same time.

Fruit: the erect, inflated berries are placed rosette-like almost above the plant. The berries are oblong conic, slightly bent, 8—12 cm long ending in a tip. The surface is smooth. The fruit when unripe is dark green, then becomes red, turning into dark red at the stage of full ripening. It does not taste hot. The dry matter content of the fruit is an average of 18.3 per cent, the pigment content (capsanthin) is 5.3 g/kg (TUZA—HORVÁTH 1973).

Seed: almost round, flattened, with a prominent basal part; diameter is 4.5—5 mm; colour light yellow. The hilum forms a shallow bay and reaches down to the beak-like basal part. Thousand-seed-weight is 4—7.2 g.

Biological characters:

Germination: When sown or planted late (beginning of June) the germinability may decrease by 30—40 per cent.

Vegetative period: sowing at the beginning of April and transplantation toward the end of May ensure the most favourable development.

Water requirement: medium; the variety is tolerant to drier conditions too; irregular water supply and fluctuating temperature cause cracks in the berries (TUZA—HORVÁTH 1973).

Resistance to disease: rather resistant to viral stem diseases, but fairly susceptible to fruit diseases; moulding during storage may even be 30—35 per cent.

Farm technology requirements:

Seeding: if optimum development is to be achieved the seeds are sown into seedling beds at the beginning of April; its short vegetation period makes direct sowing also efficient.

Planting: 333 000 seedlings per ha spaced at 60 × 15 cm in an alternating pattern, at the end of May, beginning of June. Earlier planting results in lower yield. Seedlings planted one by one develop more favourably.

Soil requirement: It yields better on heavy soil.

Productivity: Average yield 104 q/ha(fluctuation: 80—132 q/ha); on each occasion one-third of the berriers are harvested of which nearly 70 per cent are healthy (TUZA—HORVÁTH 1973). 64.6 per cent of the berry is fruit-wall; the seeds represent 19.7, the veins 8.1 per cent of the fruit (there are mostly two veins in a fruit). The peduncle is 7.6 per cent of the fruit yield.

Region of cultivation: most efficiently grown in the red pepper district of Kalocsa.

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INSECTICIDAL SPRAYINGS CAUSING POLLEN STERILITY IN CHINESE CABBAGE

The disadvantages of the ever-growing use of pesticides are now being increasingly realized. Some of the long- and short-term hazards are the insect resistance to pesticides and their toxic effects on soil micro-organisms, crop plants, wild life, domestic animals, parasites, predators, pollinators and even on man himself (Simmonds 1968, Busvine 1968, Van De Vrie 1967, Felton 1969, Lal 1969, 1971, Geier *et al.* 1967). Insecticides and fungicides are also reported to affect the pollen viability of different crops (Dhuria *et al.* 1965, Braun—Schonbeck 1963, Eaton—Chen 1969, Gentile *et al.* 1971). The present contribution reports about pollen sterility caused by various insecticidal sprayings on Chinese cabbage at blooming stage.

The insecticides used were: methyl demeton (0.025 per cent), dimethoate (0.03 per cent), parathion (0.025 per cent), endosulfan (0.05 per cent), trichlorphon (0.1 per cent), toxaphene (0.06 per cent), lindane (0.04 per cent), DDT (0.1 per cent), thiometon (0.035 per cent), carbaryl (0.1 per cent), phosphamidon (0.03 per cent), dichlorovos (0.05 per cent) and malathion (0.1 per cent). These were sprayed on a bright sunny day on the whole plant of Chinese cabbage, variety "Pe-tsai", when it first started flowering at the rate of 1000 litres per hectare. Six hrs and 1, 7, 14, 21 days after spraying flowers from plants under different treatments including control (water sprayed) were collected separately in small butter paper bags and brought to the laboratory. Pollen from all the flowers under each treatment were mixed together and their sterility was determined by the acetocarmine test under a microscope. Unstained, shrivelled and vacuolized grains were counted as sterile. In all 100 pollen grains for each treatment were counted from 10 different microscopic fields on a slide and the percentage of sterile grains was calculated.

The degree of pollen sterility produced by different insecticides is presented in Table 1. It is evident from the findings that all the insecticides affected the pollen viability. After 6 hrs, only dimethoate, parathion, phosphamidon and methyl demeton caused pollen sterility ranging from 20 to 25 per cent. Probably, the other insecticides do not possess a quick action to affect the pollen viability within 6 hrs. After 24 hrs, dimethoate, parathion, methyl demeton and phosphamidon caused 58, 51, 50 and 40 per cent pollen sterility, respectively. Lindane, malathion and endosulfan were comparatively less toxic. The other insecticides were medium for their action on pollen viability. One week after spraying, only DDT, dimethoate, parathion, methyl demeton and phosphamidon could cause more than 20 per cent pollen sterility. The pollen viability affected by different insecticides after 14 days ranged between 8 to 23 per cent and after 21 days there was no effect (Table 1). Thus, it is clear that most of the insecticides affect the pollen viability only for the first 4—5 days, except a few which may affect it even up to 12 or 15 days.

However, the pollen sterility produced by the insecticides could not be of any practical use because only more than 90 per cent pollen sterility may be practically utilized in hybrid seed production (Choudhury—George 1962). Choudhury—George (1962) reported that maleic hydrazide at 0.6 per cent, naphthaleneacetic acid at 0.05 per cent, 2.4—D at 0.02 induced 90 to 100 per cent sterility in two brinjal varieties for a period of one week,

Table 1

Effect of some field sprayed insecticides on the pollen sterility of Chinese cabbage

Insecticides	Percentage of pollen sterility after				
	6 hrs	1 day	7 days	14 days	21 days
Lindane	7	22	20	10	2
DDT	9	33	24	17	3
Dimethoate	25	58	48	23	5
Malathion	5	23	14	11	3
Carbaryl	10	35	14	12	1
Endosulfan	8	21	18	12	4
Parathion	25	51	35	19	2
Methyl demeton	20	50	26	17	3
Phosphamidon	20	40	35	17	4
Dichlorovos	11	27	16	13	3
Thiometon	15	30	15	12	8
Trichlorphon	12	25	17	13	5
Toxophene	8	26	13	8	4
Control	5	4	6	4	5

14 to 28 days after spraying. But, the insecticides under present investigation did not affect the pollen viability after 21 days. However, the mode of action of growth regulators and insecticides are quite different which is probably responsible for the contrasting results. Possibly, the insecticides produce abnormal cell elongation in the anthers due to which a number of pollen grains lose their viability.

Gentile *et al.* (1971) studied the effect of some formulated insecticides at 100 ppm actual material on the germination of petunia and tomato pollen on artificial media and found about 88, 69, 50, 48, 20 and 9 per cent inhibition by the topical application of parathion, endosulfan, dichlorovos, DDT, carbaryl and control, respectively, after 15 hrs. The pollen sterility produced by different field sprayed insecticides, under present investigation, was comparatively much less than that reported by Gentile *et al.* (1971), which they found after topical application of insecticides on the artifical media. This is, probably, due to the fact that the morphological structure of the floral parts may prevent a large amount of pesticides from coming into contact with the pollen. Moreover, they used higher concentrations, which may be another reason for higher pollen sterility. Gentile *et al.* (1971) found endosulfan more toxic than dichlorovos but after field spray it was found that endosulfan was somewhat less toxic to the pollen grains than dichlorovos which is probably due to the quick degradation of endosulfan under field conditions.

The effect of insecticides in case of insects as reported by Sharma—Chattoraj (1964) causes a general shrinkage and vacuolization in all the affected tissues. Since most of the plants can tolerate insecticides more than insects, as at normal doses only the insects are killed without phytotoxic effects, it is possible that only the anthers which are very delicate parts of the plant become the physiologically selective site for the action of the insecticides, and due to expulsion of water they shrink and the viability of the pollen grains is lost.

In case of dimethoate, one day after treatment the bursting of the pollen grains was observed under a microscope and most of the cells lost their protoplasm. The shedding of the cytoplasm and disappearance of cell contours due to rogor proves that amongst all the insecticides used in this experiment rogor is the most toxic for the pollen grains of Chinese cabbage. Though rogor is accepted as a good insecticide because it kills most of the insect pests parasites, predators and pollinators, it also causes pollen sterility to a considerable extent which may be quite harmful for self and cross pollinated crops. However, malathion, lindane and endosulfan are comparatively less toxic to the pollen grains.

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WHAT CAN THE "NEW METHOD FOR THE RAPID DETERMINATION OF AUXIN CONTENTS" BE USED FOR?

The auxin routine test method briefly described in Acta Agronomica (Brunner—Antoni-Gál 1971a) has been remarked on by Sági (1972). On this occasion I think it right to summarize the views and aims I was led by in elaborating this test method, and give information at the same time on the possibilities of application and the results attained so far.

I had practical considerations in the first place, so it is quite natural that this routine test cannot satisfy the highest biochemical demands, but it is all the more suitable for interpreting physiological phenomena arising in the course of agrotechnical research, solving preselection problems and — in general — for disclosing numerous causal relations.

Besides its simplicity and rapidity the method has the advantage of eliminating a number of steps involved in obtaining auxin by diffusion of extraction, and preventing at the same time a possible related denaturalization and other defects. The use in biological tests of auxin obtained from a plant material has another element of unreliability too, namely that it has to induce the growth response from which we can conclude on its quantity in a different type protein. On the other hand, errors naturally following from the character of the routine test are generally compensated by the fact that in the auxin determination of two or more samples these errors appear parallel and so the comparative quantities reflect realistic differences (Brunner—Antoni 1970). This statement is supported by the following results:

1. With a view to elaborating the preselection method of fruit-tree stocks we examined one-year old cherry *(Cerasus avium)* and sour cherry *(Cerasus vulgaris)* seedlings as well as wild apricot *(Armeniaca vulgaris)* and myrobalan *(Prunus cerasifera* convar. *myrobalana)* seedlings. In agreement with experiences gained in practice the investigations showed growth vigour differences in these species even at this early stage of development, that is, in auxin content simply expressed in extinction sour cherry was exceeded by cherry and wild apricot by myrobalan (Brunner—Antoni-Gál 1971b, c).

2. However, it was not only at the juvenile but also at the generative stage of the material that real differences of auxin contents as expressed in extinction were obtained. When examining shoots of true-bred varieties at the same phenophase we found that in accordance with the pomological descriptions (Brózik—Regius 1957) in Nyári Kálmán qualified as of intensive growth habit, the medium strong Hardenpont and the relatively weak Bosc pear varieties the auxin contents of lignified shoots corresponded to the mentioned qualifications. The same held true of the auxin content of shoot formations being higher in the vigorous apple variety Starking than in the less vigorous Jonathan (Brunner—Antoni-Gál 1971b).

3. True correlations were also showed between the activity of the stem thickening cambium in apricot, cherry and sour cherry shoots on the one hand, and auxin content determined by the routine test, on the other. Shoot formations of apricot were tied down at the stage of shoot and cane, respectively. As a result of tying down canes the diameter of the cane decreased by 28 per cent compared to the upright control, and by the end of August its auxin content was 14 per cent lower too (Brunner 1972a). An even closer correlation between the activity of the cambium and the extinction value of the auxin content determined by the routine test was disclosed by a serial investigation when upright shoots of the indeterminate Germersdorfi cherry and Pándy sour cherry varieties were studied on 14 occasions throughout the vegetation period, and at the end of the growth season the results were compared with the diameters of similar cherry and sour cherry shoots left on the trees. When averaging the auxin levels obtained on the 14 occasions we found that the auxin content was 17 per cent higher at the base, and 38 per cent at the apex in the Germersdorfi shoots than in the corresponding parts of the Pándy shoots throughout the whole vegetation period. This average auxin level difference shown at the apex was excellently reflected in the shoot diameters measured toward the end of September (on shoots left on the trees), namely, the shoot diameter of the Germersdorfi cherry variety exceeded that of the Pándy sour cherry variety by 45 per cent. This proves that the relative percentage difference of auxin levels determined by the routine test only deviated from the relative percentage difference of the shoot diameters measured at the end of the vegetation period by 7 per cent. In addition, the average shoot length measured at the end of the growth season was nearly identical in the two materials (Brunner 1972b).

4. Serial auxin routine tests performed during the vegetation period with peach shoots treated with gibberellin in spring showed that under the influence of gibberellin the auxin content in September was 21 per cent higher, and at the same time the final length of the shoot was 18 per cent greater compared to the untreated control. In July and August, on the other hand, when the growth of the treated shoots was 16—22 per cent faster than that of the untreated

control, the auxin level was temporarily 24—32 per cent lower in the former group probably due to an increased auxin utilization involved in the acceleration of growth (LŐCSEI—BALÁZS—BRUNNER—DVORSCHÁK 1973).

5. In the auxin content of seeds from wild apricot *(Armeniaca vulgaris)* and myrobalan *(Prunus cerasifera* convar. *myrobalana)* stored together with apple for three weeks at room temperature a 22—23 per cent reduction was pointed out by the routine test. That is, the hormonal effect of ethylene, as a gas stimulating the decomposition of auxin was very quickly and simply demonstrable (BRUNNER—ANTONI-GÁL 1973).

The above results are exclusively based on auxin contents expressed in extinction. This is the simpler solution, and it is not sure at all that we can obtain a more realistic picture by calculating the actual auxin content on the basis of the calibration curve. Namely, IES when directly dissolved in Gordon-Weber quantitatively does not dissolve proportionately, so we can only work indirectly: with a calibration curve made on the basis of various concentrations of alcoholic IES solution.

In short, the auxin routine test is suitable for determining the growth vigour both at the juvenile and generative stage during the pre-selection work of breeding, and at the same time is able to reflect certain characteristic changes of the auxin turnover occurring as a result of agrotechnical interventions or other treatments and actions. For this very reason the auxin routine test can — in my opinion — be applied in many fields where rapid and detailed information is required concerning the whole or certain parts of the plant, for example when developing and evaluating new varieties or root-stocks, or elaborating new agrotechnical methods.

Finally, I give here a short description of the auxin routine test:

a) An adequate quantity (e. g., 1—2 g) of the material cut up to pieces of 2—3 mm size is extracted with 10 ml 35 per cent perchlorate, and the same quantity with 10 ml Gordon-Weber reagent over one hour at room temperature, while stirred from time to time.

b) Both solutions are filtered, and 5 ml of each measured into cuvettes for photometric purposes. The perchlorate solution is zeroed to 35 per cent perchlorate, the Gordon-Weber solution to Gordon-Weber reagent.

c) Extinction is measured by an UVIFOT or other photometer at the wavelength IA (510 nm).

d) The extinction of the perchlorate solution is subtracted from the extinction of the Gordon-Weber solution. The difference of the two will give the extinction produced by the Gordon-Weber reagent under the influence of the given auxin content. The extinction of the perchlorate solution subtracted from the extinction of the Gordon-Weber solution ensures the elimination of the extinction of natural colour substances extracted together with the auxin, and thus the precisity of the analysis.

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WOOD ANATOMY-XYLOTOMY

Importance of wood as raw material. In the world of to-day, besides oil, coal, natural gas, water, etc., wood is also one of our most important raw materials that cannot be dispensed with either in manufacturing buildings, furniture, matches, wood-fibre, cellulose, artificial silk or in tool-engineering, paper-making or even, we could almost say, in fine art. We should just think of the different musical instruments made of wood, wooden fancy articles, wood sculpture or the world-famed pictures painted by distinguished artists upon wood, etc. Apart from all these, the archaeologists too, often find during their excavations prehistoric remains of wood, e. g. hearths, tools, domestic utensils of the primitive man, coffins, barrels, building and furniture remains, etc., the identification of which is first of all the task of botanists who know the structure of the different woods. Then from the identification of wood remains the historian of civilization can draw valuable conclusions.

A rather exact knowledge of prehistoric woods has a very high scientific value in another direction, as well. Of late, a greater and greater number of the remains of trees that had lived in a bygone period have been excavated from the depth of the Earth, from mines. They are sometimes only small, fine wood shreds, at other times, however, they are larger or smaller branch pieces of former terrestrial plants and later on they can get as tree-trunks of fossilized woods or their pieces under the pickaxes of miners. The different branches of science, first of all palaeontology, phylogeny, systematics, phytogeography, climatology, geology, and even petrography want to know under which conditions these ancient land-plants and woods may have lived many hundreds of thousands or millions of years ago and owing to what possible catastrophes (forest-fires, volcanic eruptions, etc.) they may have got possibly several hundred metres deep into the depth of the Earth. As these former terrestrial plants, in their individual life, had some particular organization and structure preserving them in the depth of the Earth, too, during the millions of years, in this way the different branches of science can ascertain far-reaching scientific and practical relations from the identifications of these fossilized wood relics.

And the results of wood investigations are available not only for the historian of civilization, the palaeontologist but also for the forester, craftsman, technician, and even often for the legal courts, too (we should remember the kidnapped Lindberg-baby).

Wood is very important as fuel, as well. In winter, as we kindle a fire in our stove with fir-wood sticks and then put on the embers logs of hornbeam, oak or beech wood, not one of us thinks how many and interesting things we consume by fire by burning a small firelog like those. If, anyway, we had X-ray eyes magnifying in these cases at least one hundred times and could observe the ends or sides of the X-rayed wood logs, we could notice inside the wood great, interesting, natural and artistic beauty. As the inside of wood is not visible to the naked eye, we have to ask for the help of science to get an inside view of the life and beauty of trees. First, however, we have to interrogate both trees and woods. To be sure, both trees and woods are dumb but they speak loudly to those who have learned their language.

Wood anatomy — wood histology — xylotomy. This language is to be deciphered by the several kinds of institutes for forestry and wood research established in the various countries for exploiting wood as economically as possible. Namely some countries obtain a great part of their national income just from wood as raw material. Here we should think, e. g., of the highly developed wood industry of Canada or Finnland. This multilateral interest in the raw material of wood has gradually developed several particular branches of science dealing with wood as, for instance, forestry, wood pathology or xylotomy. Its great importance is shown by the fact that to-day the scientific investigation of woods already has a great international organization, as well. This purpose is served by the International Association of Wood Anatomists, having meetings in every fifth year as a sub-department of the International Botanical Congresses in the various countries where the wood-scientists of the world give information about the most recent results of their wood investigations. The number of its members is cca 200. The author is also a member of this international association. Their central periodical is: Bulletin: International Association of Wood Anatomists (IAWA). State University of New York.

The science dealing with the inner finer structure of woods is called wood anatomy, wood histology or xylotomy. It is extremely interesting that every kind of wood contains components, cells that are characteristic of the wood in question. In this way, we can identify the sort of wood even from a tiny piece of it, e. g., from a toothpick made of that wood.

With the help of science, at any rate, we can also investigate the structure of trees that had lived many hundreds of thousands or millions of years ago and even that of coals. With the help of a microscope and knowing the structure of wood we can say for what purpose some wood may be used the most properly and suitably. (We should think of the material of the renowned Stradivarius violins.)

The method of xylotomy. Preliminary study. Xylotomy as every other branch of science has its own method and language. Therefore, a rather exact knowledge and identification on a histological basis of any tree-stem or piece of branch, either the trunk of a tree or that of a fossil wood, can be performed with success only if somebody is well aware of the components of structure of the various trees living even to-day, of their arrangements in space, of the most important elements, technical terms, and if he knows that the characteristic of any living or perished tree species is determined first of all by the shape, size, and spatial arrangement of their components. Any arrangement of the components of wood is always and in every wood body characteristic and specific, shows an almost artistic design that can sometimes be observed even with the naked eye, possibly with a hand magnifying glass.

A collection of expressions used in wood anatomy has been published by the International Association of Wood Anatomists in a separate book: "Multilingual Glossary of Terms Used in Wood Anatomy", in English, French, German, Italian, Portuguese, Spanish and Croate-Serbian languages. The same aim is followed by P. Greguss's work: „Einführung in die Paläoxylotomie" in which the nomenclature is illustrated with suggestive pictures, as well.

Cuttings, slides, polished samples. An investigation of the inner and finer structure of woods is, however, only possible by the means of a microscope and thin cuttings, resp. polished samples made of the woods concerned. The cuttings made of trees or polished samples, slides made of fossil woods reveal under the microscope the full tissue structure of woods, in this way even the finest details characteristic of the species of the one-time tree. If, therefore, we want to know the inner structure of a living tree or of a fossil wood piece, e. g., of a lignite, then the very first step to that is to prepare cuttings, resp. slides, thin like a newspaper, of the living tree or the fossilized wood piece, in three directions as compared with the tree axis. One of them, the so-called cross section, polished sample, is perpendicular to the longitudinal tree axis (in the Plates marked with C—C = cross section), another that goes through the axis and the diameter, resp. tree ray is the radial section, resp. polished sample (in the Plates marked with R—R = radial section), and a third one made parallel with the axis but in a tangential direction. (In the Plates marked with T—T =tangential section.) For identifying a living tree or fossil wood it is not enough to have only one or two kinds of cuttings or slides because on the basis of a single picture, e. g., that of a cross section, we can only identify the type which the tree in question belongs to (cf. Plates V—VI). To be sure, sometimes it is enough to have a single polished sample or cutting for beginning the further investigations in the right direction.

Four archetypes of trees and fossil woods. The whole present surface of our Earth, and the terrestrial areas of ancient ages, as well — at least since the Carboniferous period about three-hundred million years ago — has become — and is also to-day — populated only with four sorts of tree types. In this respect the oldest gymnospermous trees containing seeds were the *Gymnospermae* as the Cycads generally living in the tropics, as well as the *Coniferae* widespread first of all in the northern hemisphere. Later on, in the Quaternary of the Earth there appeared at the same time the two main types of *Angiospermae*, the *Monocotyledons*, e. g., palms, and *Dicotyledons*. Each of these four types has such a particular and different interior structure, xylotomy, that they can be easily separated from each other both in live and in fossil forms. The internal structure of these existing four types of trees is perspectively illustrated in the original drawings of Plates I, II, III and IV.

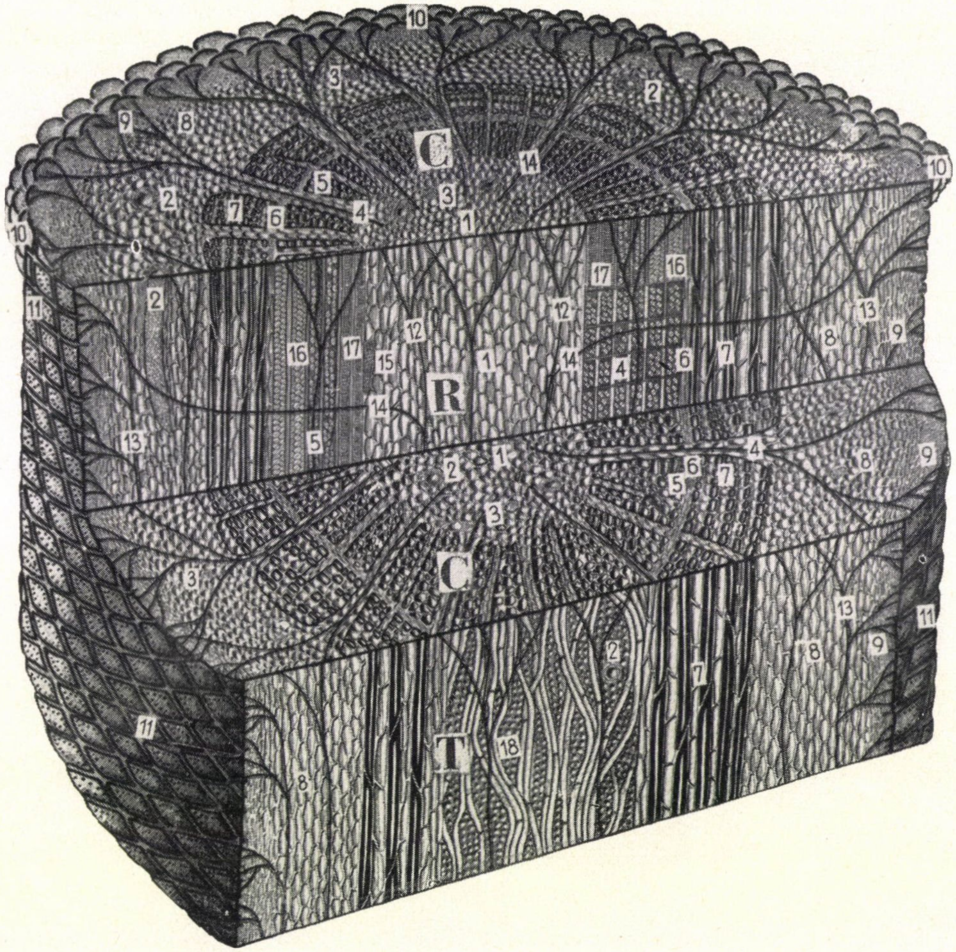
I. *Cycas*-type (Plate I). If in the cross-section (C—C) after the central developed pith (1) one or two xylem rings (6) and phloem rings (7) follow, and if in the pith and cortex there are (8) major mucilage ducts (2) and in the woody part rays (4) of two or more layers and in the rays vessels (14) then a piece of trunk like that can only belong to the Cycads. (For a more detailed description and the photographs of Cycads see later, p. 153.)

II. *Coniferae*-type (Plate II). If in the sectional picture the cross-section of the single elements, the tracheids are equal in size and arranged in a radial direction, close to one another in regular lines and annual sectors (Plate II, 7, 8 and Plate V, Figs 2a and 2b), if among these wood elements there are rays of a width of one cell layer or two going in a radial direction (Plate II., Figs 12—13 and Plate V, Fig. 2b), and in the cortex and tree body (Plate II, Figs 2, 3 and Plate V, Fig. 2b) possibly resinducts (Plate II, Fig. 15 and Plate V, Fig. 2b), then from a structure like that a sure conclusion can be drawn to a kind of *Coniferae* (Plates II and V). (A more detailed xylotomy of the wood body of *Coniferae* will be referred to later on, p. 154).

III. *Palm*-type (Plate III). If in the cross-section, in the basic substance of the wood pith (C) there are scattered larger cell groups or, more exactly, collateral closed vessel bundles, (Plates III, VI, Figs 3a and 3b), and if in the wood there aren't either distinct rays or resin ducts (Plate III), then that wood must be palm-type. (A more detailed xylotomy of Monocotyledons will be exposed later, cf. p. 154.)

IV. *Dicotyledonous*-type (Plate IV). If in the sectional picture (Plate IV. C—C), beside the minor cavities there are much larger cavities, as well — the cross sections of vessels — irre-

Plate I

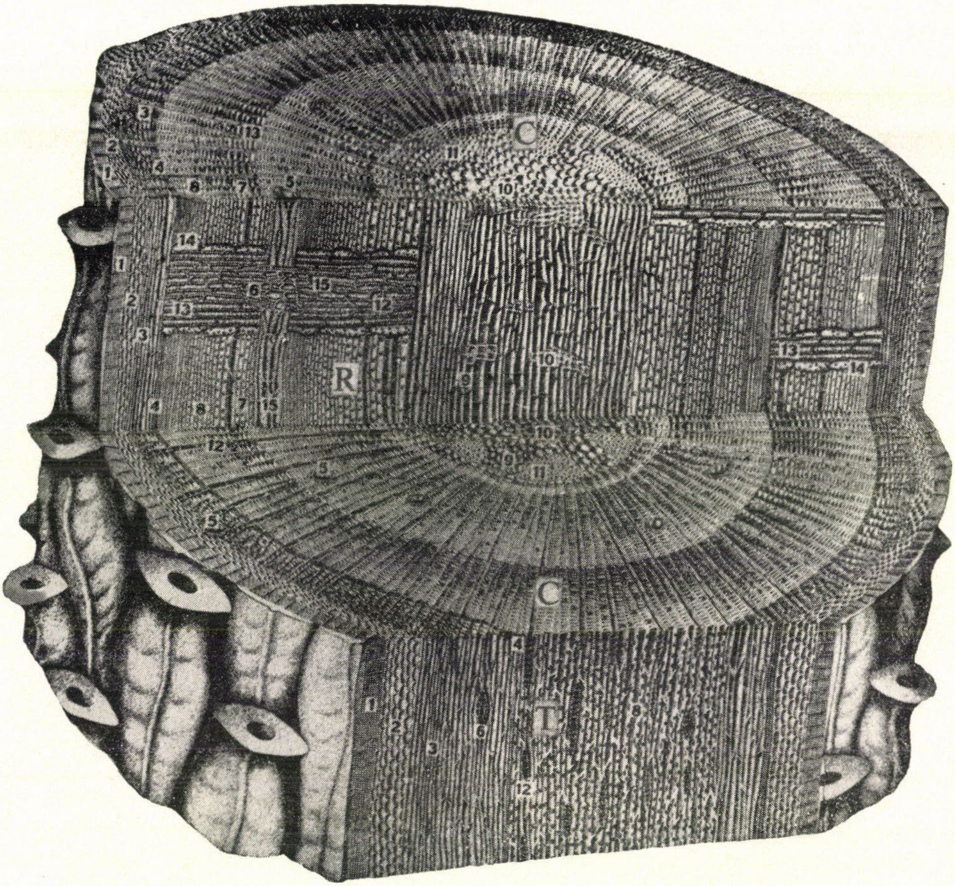


Schematic drawing of a *Cycas* stem in three planes of section. C = cross section, R = radial section, T = tangential section. 1. Pith, 2. Mucilage canals, 3. Calcium oxalate druses, 4. Primary ray, 5. Xylem part. 6. Cambium, 7. Phloem part, 8. Cortex, 9. Periderm, 10. Vestiges of leaf bases, 11. Leaf scars, 12. Pith bundles, 13. Bundles in the cortex are passing out to the leaf bases, 14. Common bundles are passing out through the primary rays into the leaves, 15. Transfusion cells, 16. Tracheids with araucaroid pittings, 17. Tracheids with scalariform thickening, 18. Multiseriate rays. (Original, Greguss and Havas.)

gularly or regularly arranged in the ground tissue alone or in smaller or larger groups, and if in the wood there go annual rings as well as rays of one layer or more arranged in a radial direction, then from that structure we must conclude on a kind of *Dicotyledons.* (The finer structure of the *Dicotyledons* will be recognized later on, p. 156.)

If we can identify an unknown wood on the basis of its sectional structure with one of the four wood types mentioned above, then this initial result may already give a valuable direction for the further more detailed and exact investigations.

Plate II



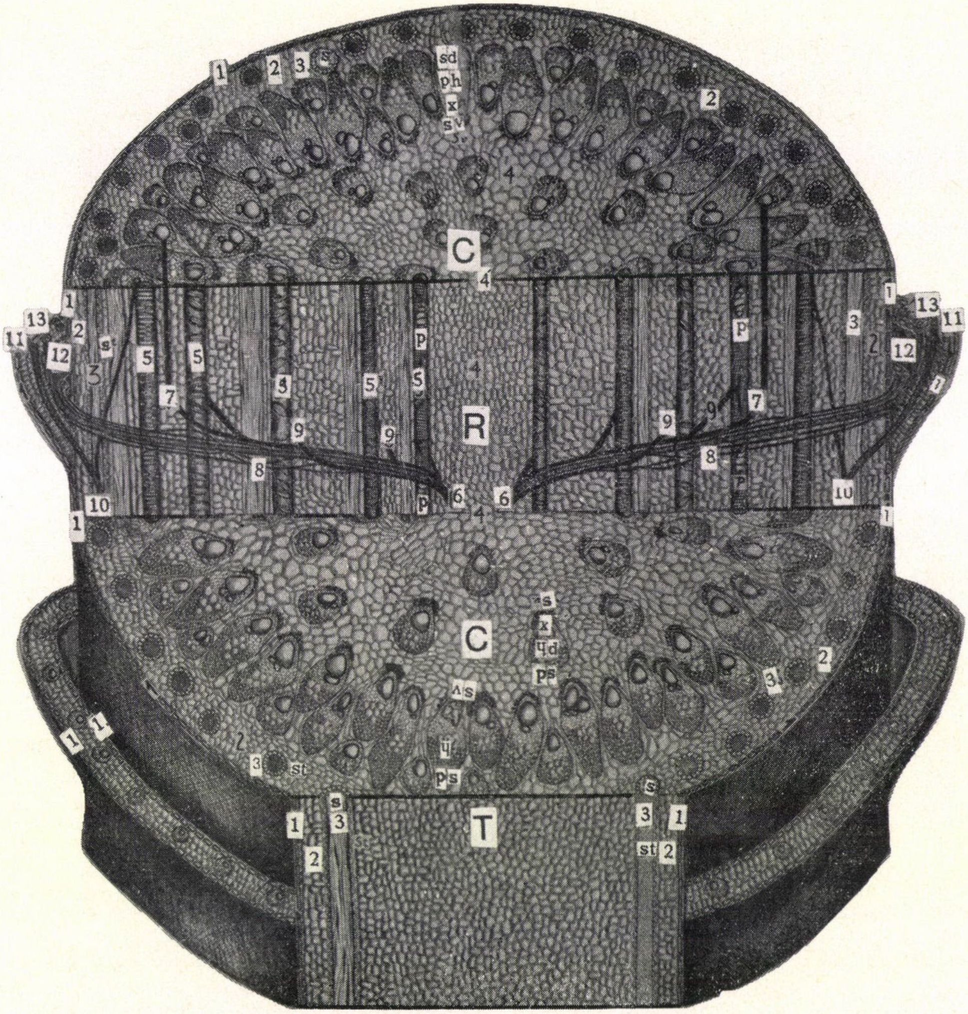
Schematic drawing showing the wood of a three years old spruce *(Picea)* twig in three planes of section. C = cross section, R = radial section, T = tangential section. 1. Epidermis, 2. Periderm, 3. Phloem, 4. Cambium, 5. Vertical resin duct, 6. Horizontal resin duct, 7. Earlywood, 8. Latewood, 9. Pith, 10. Pithsclerenchyma, 11. Primary wood, 12. Medullary ray (seen in tangential view), 13. Thick-walled way cells, 14. Marginal cells, transverse tracheids, 15. Thick-walled epithelial cells. (Original, Greguss and Gosztonyi.)

It is very natural that even inside the existing four main wood types there is an extremely great variety. Nevertheless, these four kinds of anatomic type marks appear in each of the families and species. In this way, not only the single families but also the species can be well separated from each other and identified.

The most characteristic xylotomic peculiarities of wood body of the different wood types

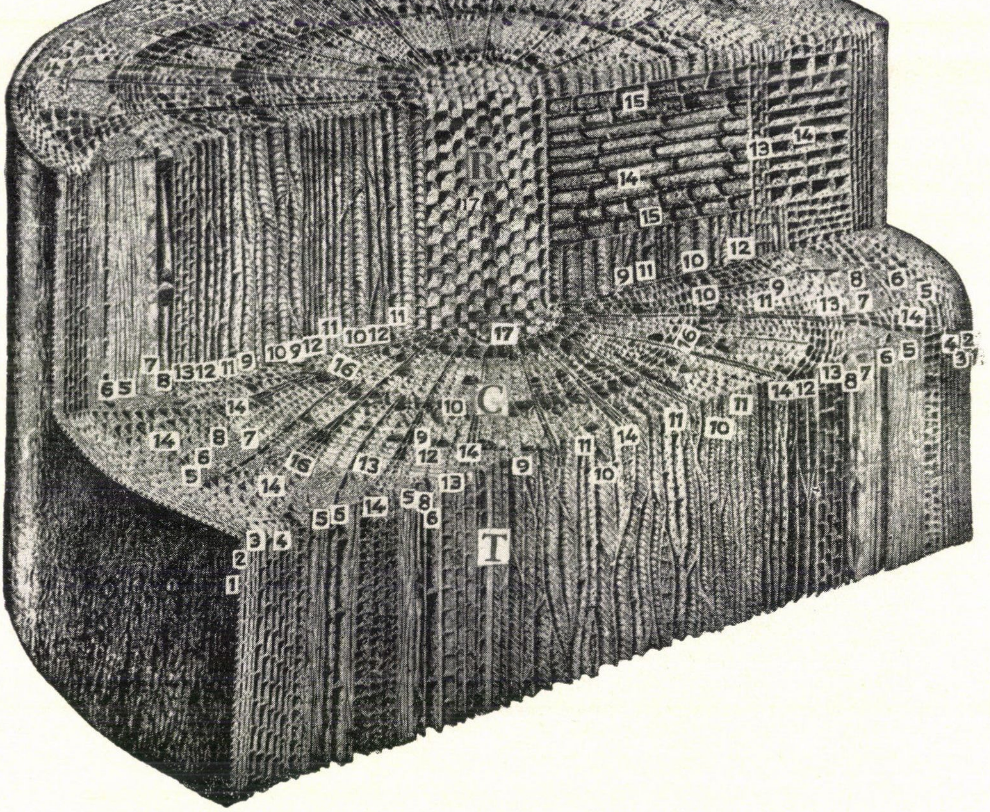
I. Wood structure of Cycads (Plates V, 1a, 1b and VII). The Cycads living to-day can be divided into ten genera: 1. *Bowenia*, 2. *Ceratozamia*, 3. *Cycas*, 4. *Dioon*, 5. *Encephalartos*, 6. *Lepidozamia*, 7. *Macrozamia*, 8. *Microcycas* 9. *Stangeria*, 10. *Zamia*. There are about 140

Plate III



Schematic drawing showing the wood of a palm stem *(Raphis)* in three planes of section. C = Cross section, R = radial section, T = tangential section. Cross section, 1. Epidermis on the stem and in the leaves, 2. Parenchyma cells of the cortex. 3. Sclerenchymatous fibres (Sc) with stegmata (St). In the ground tissue the vascular bundles are sporadic. Parts of the vessels: X = Xylem, Ph = phloem, Sd = dorsal sclerenchyma, Sv = ventral sclerenchyma. Radial section. On the side of stems there are two leaf bases. Upper and lower epidermis. (1—1). In the middle there is the mesophyll. The sclerenchyma fibres are with stegmata. The veins are travelling in the leaves. In the vascular bundle there are vessels with scalariform and spiral thickenings. (5). P = perforation. The black and hachured line shows the running of vessels. (6). From the mark spall the big bundles on the one side, the vertical bundle (7—7) into the satellite bundle (8), after into the bridge, and go out in the leaves (11) and in the cortex. (7). In the outer cortex little bundles come (10) from under upwards and pass out as leaf base bundles in the leaves. Tangential section. 1. Epidermis, 2. Cortex parenchyma, 3. Sclerenchyma fibres (Sc) with stegmata. (Original, Greguss and Meskó—Bóka.)

Plate IV



The internal structure of a two years old twig of lime-tree *(Tilia)*. C—C = Cross section, R—R = radial section, T—T = tangential section. 1. Cuticula, 2. Epidermis, 3. Bark, 4. Bast, 5. Bast fibres, 6. Bast parenchyma cells, 7. Companion cells, 8. Sieve tubes, 9. Tracheids, 10. Vessels, 11. Wood parenchyma cells, 12. Fibre tracheids, 13. Cambium, 14. Rays, 15. Rayedge cells. 16. Annual ring border, 17. Pith. (Original, Greguss and Tóth.)

species of these ten genera, however, they differ from one another in their external shapes and the development of their blooms, showing several common and characteristic features in their xylotomy in which they differ essentially from the other tree types, first of all from the other gymnospermous-type, the *Coniferae.*

Cross-sectional structure. One of the most characteristic cross-sectional peculiarities of every genus and species of the Cycads is that they contain in their central part a well separated pith consisting of parenchyma cells (Plate V, Fig. 1a). The pith is permeated, as a rule, by mucilage ducts (Plate V, Fig. 1a, Plate VII. Figs 2, 6) that generally communicate with similar mucilage ducts of the cortex (Plate I, Fig. 8), through the multiseriate rays (Plate I, Figs 4, 14, Plate V, Fig. 1b). In the pith of most Cycads there are among their own bundles also some so-called common vessel bundles (Plate I, Fig. 14) passing from the pith through the multiseriate rays into the cortex and from there as real leaf traces into the leaves. The

Plate V

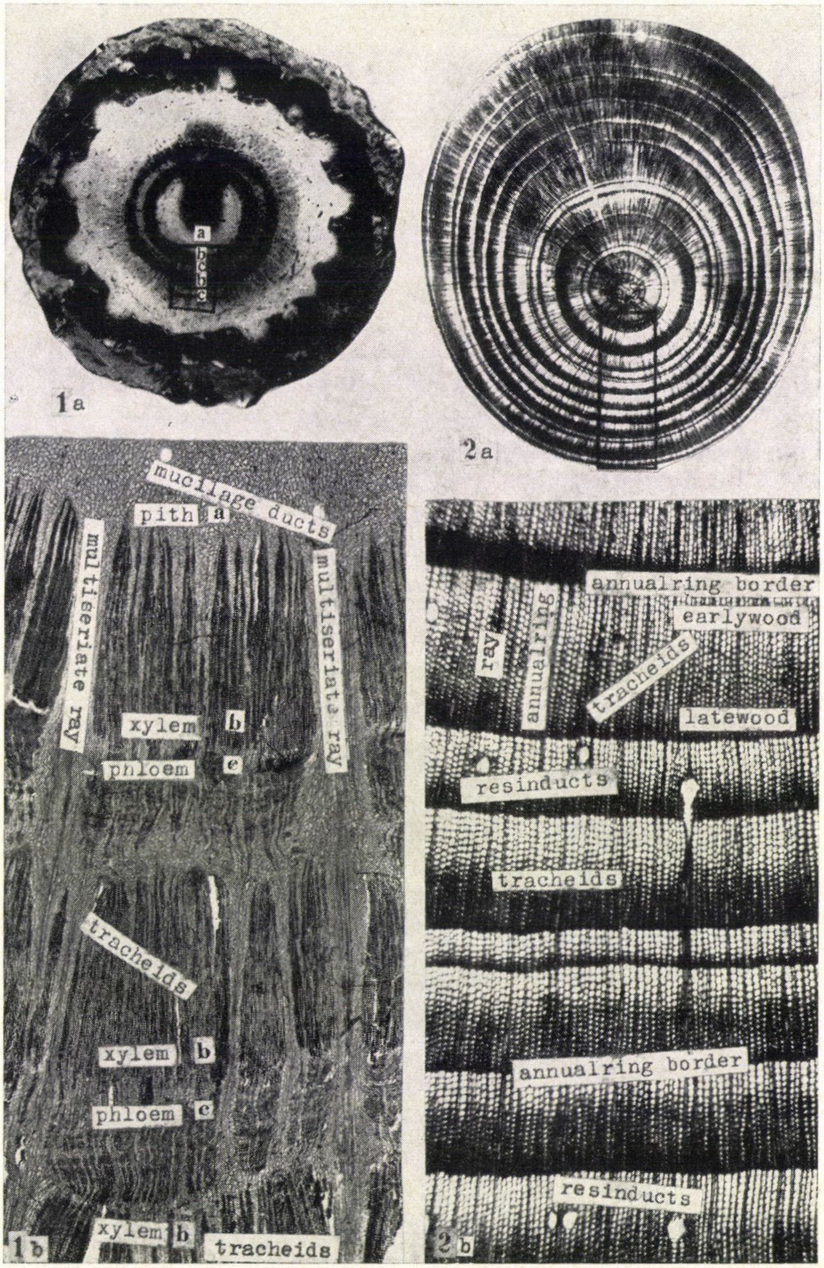


Fig. 1a. Cross section of *Cycas revoluta* 1/2 nat. size, a) pith, b) xylem part, c) phloem part. *1b.* Internal structure of conductive bundles, (×15). *2a.* Cross-section structure of a 12-year-old twig of spruce (Picea). *2b.* Anatomical cross-section structure of the spruce (×30). (Original.)

Plate VI

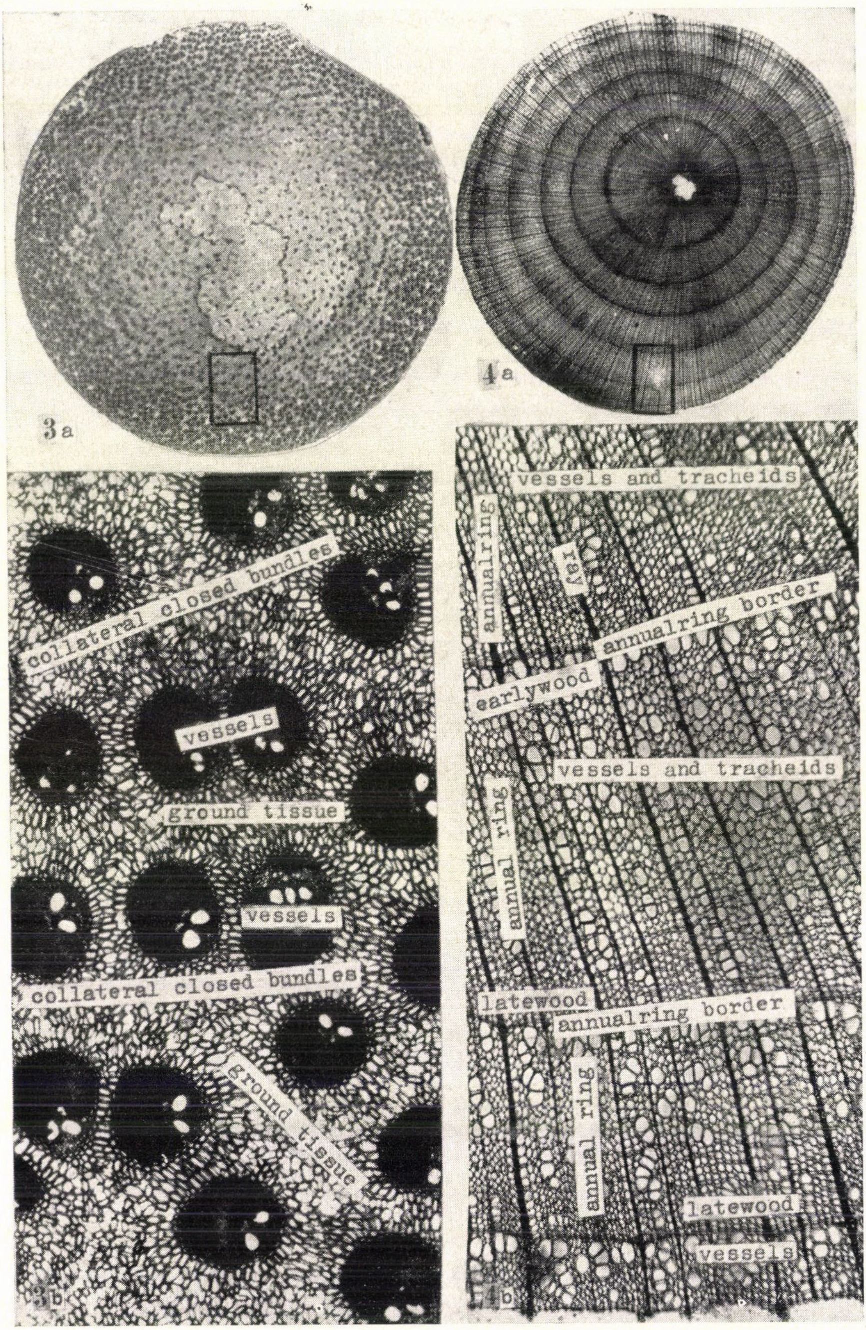


Fig. 3a. Cross-section structure of a palm stem (*Raphis*, ×30). *3b.* Anatomical structure of a palm stem (Raphis, x30). *4a.* Cross section structure of a dicotyledonous tree. (*Tilia*, ×5). *4b.* Anatomical structure of a dicotyledonous tree (*Tilia*, ×50). (Original.)

collateral vessel bundles (Plate I, Figs 6, 7) wind about irregularly both in the pith and in the cortex, in some places they pass parallel to the axis (13), others are, on the other hand, almost perpendicular to it.

Another peculiarity of the Cycads is that the large pith is surrounded 1—8 times by a xylemand phloem-ring (Plate I, Figs 6, 7, Plate V, Figs 1b, b—c, b—c). In the wood body the tracheids (Plate I, Figs 16, 17, Plate V, Fig. 1b), are mostly arranged in a radial direction, similarly to the trees of *Coniferae* (Plate VII, Figs 3, 4). In the wood body, the annual rings cannot be separated as in the *Coniferae.* The rays are uniseriate only in the rarest cases, mostly they are even 2—10—15 cell-layers wide (Plate I, T. 18). These latter are the multiseriate rays (Plate V, Fig. 1b), going immediately from the pith towards the cortex. This multilayer wide ray structure is definitely a Cycas characteristic, in this way conspicuously differing from the structure of the wood body of any Coniferae. Every wall of the ray cells is smooth and fully thin, even simple pits can be observed in them only very scarcely (Plate VII, Fig. 11). Beside the xylem ring (Plate I, Fig. 5) there is always phloem ring and between them a cambium of one cell layer or two can exist, as well (Plate VII, Fig. 3). In the phloem part we can sometimes well distinguish the phloem parenchyma from the phloem fibre (Plate VII, Fig. 3) and sometimes also from the sieve tubes perforated with tiny holes.

Beside the phloem components and always close to the wood part frequently there are transfusion cells (Plate I, Fig. 15, Plate VII, Figs 2, 5, 8) that are thickened, bordered pit-like or networklike. In some cases it is obvious that these transfusion cells are in fact protoxylem elements, being isodiametric, and the development of long tracheids can be followed from them very exactly.

Tangential structure (T). The rays are of different height and width. In this respect they differ from the rays of *Coniferae* (Plate I, Fig. 18, Plate VII, Fig. 7).

On the radial side (R), too, there are many characteristic peculiarities. The most important mark is that the ray cells are nearly always in a standing situation (Plate VII, Figs 11, 12), and in the crossfields the single pit series are arranged not in horizontal but in perpendicular lines and, as a rule, in an araucaroid way (Plate I, Fig. 16, Plate VII, Fig. 12). The pit aperture is mostly of horizontal site or somewhat oblique. In genera containing only tracheids (Plate I, Fig. 17, Plate VII, Fig. 9), e. g. in the Zamiaceae, there are no characteristic cross fields.

Another characteristic of the genus is that the tracheids contain pits. In some tracheids there are only pits of an araucaroid type (Plate I, Fig. 16, *Encephalartos*, Plate VII, Fig. 10), in others, however, the thickening of the tracheids has a completely fern-like character, i. e., it is ladder-like (Plate I, 17, Plate VII, Fig. 9), again in others transitory forms can be observed to a network-like thickening. If we can notice in a wood xylotomic marks like these it must belong to the Cycads and then the detailed investigation may have the identification of species as its aim. (A more detailed investigation of the *Cycadaceae* living to-day can be found in P. Greguss's book. See: References.)

II. *The most characteristic xylotomic features of Coniferae* (Plates II, V, and VIII). Both the living and the fossil *Coniferae* trunks have some xylotomic features in which they considerably differ both from Cycads and from the monocotyledonous and dicotyledonous woods. Plate II shows a three-dimensional wood structure of a three-year old spruce, more exactly of *Picea excelsa.* The other *Pinaceae*, living to-day or extinct, have a largely similar structure to that.

Round the axis of the young wood body, the pith is formed by tissue components of rather thick walls (9) sporadically containing pith sclerenchyma cells with thick walls (10). The pith is permeated in some places by the wood elements of primary vessels, these form the primary xylems (11). Outside the pith, the concentric circles are the annual ring borders (Plate V, Fig. 2b), and between them the annual rings. In the annual rings we can separate a zone of early wood from that of late wood (Plate VIII, Figs 2a and b). The wood body is interrupted by radially passing cell plates, rays (Plate II, Fig. 6). Inside the rays, the parenchyma cells are prolongated in the ray direction, those in the middle differ in structure from the upper and lower lateral cells, these being the ray tracheids (Plate II, Fig. 14). In the wide rays there rather frequently pass horizontal resin ducts that are connected with the perpendicular resin ducts. The resin ducts of Picea are lined with thick walled epithelial cells (Plate VIII, Fig. 5). Between the xylem and the phloem, on the surface of the wood body, there is a cambial zone, outside it being the phloem (Plate II, Fig. 3), followed by the cortical parenchyma (2), while the most outer part the young branch is covered with a cork cortex developed in the site of the epidermis.

Figs 2a and 2b of Plate V present the cross section of a several-year-old Picea branch (magnified × 5 and a detail of it 5b magnified ×50), with the proper nomenclatures. In Plate VIII, on the other hand, we see the finer structure that is characteristic of the *Coniferae.* The wood structure of the different *Coniferae* is, of course, very much varied both in cross-sections and in tangential and radial sections. In the first column we see the various cross-sectional structures. In Fig. 1, the cross sections of tracheids are rounded off, and in the section there aren't any conspicuous annual ring borders *(Araucaria).* In Fig.2, there appear striking annual ring borders *(Abies)*, and in the annual rings wood (a) and latewood (b) can be separated. Fig. 3 presents resin-cysts that are not real resin ducts *(Abies).* In Fig. 4, the wall of the epithelial cells of the resin ducts is thin *(Pinus silvestris).* At the bottom of Figure 5, the wall of the epithelial cells of the resin ducts is thick *(Picea).*

In column 2 we can see tangential structures. Fig. 6. Uniseriate high rays *(Abies).* Fig. 7. Rays with resin ducts *(Pinus).* Fig. 8. Juniperoid pit in the tangential walls of ray cells *(Cupressus).* Fig. 9. Simple pit *(Abies).* Fig. 10. Heterogeneous ray skeleton *(Pinus).* Fig. 11. The end-walls of longitudinal parenchyma cells are smooth *(Taiwania).* Fig. 12. A wall thickened like a necklace of pearls *(Juniperus).* Fig. 13. A wall thickened like a cog-wheel (*Taxodium*). Fig. 14. Very high ray *(Taxodium).*

Different cross-fields of rays. Fig. 15. Pinoid pit (*Pinus*). Fig. 16. Taxodioid pit *(Taxodium).* Fig. 17. Cupressoid pit *(Cupressus).* Fig. 18. Podocarpoid pit (*Podocarpus*). Fig. 19. Dacrydioid, circoporous pit *(Dacrydium).*

III. *Xylotomic characteristic of monocotyledonous trees* (palms) (Plates III, VI, and IX). One of the representatives of the *Monocotyledons* is *Raphis flabellaris* (Plate III). The cross-sectional structure of palms differs considerably from both tree-types mentioned above first of all as palms have atactostele, i. e., the single collateral closed vessel bundles are arranged irregularly in the ground tissue (Plate VI, Figs. 3a, 3b). The single bundles are closed or semi-closed (Plate IX, Fig. 2, Plate VI, Fig. 3b). The single vessel bundles are generally surrounded by sclerenchyma fibres. In the outer part of vessels there are Sclerenchyma dorsale (sd), and in the axis region Sclerenchyma ventrale (sv). Apart from them there is the xylem

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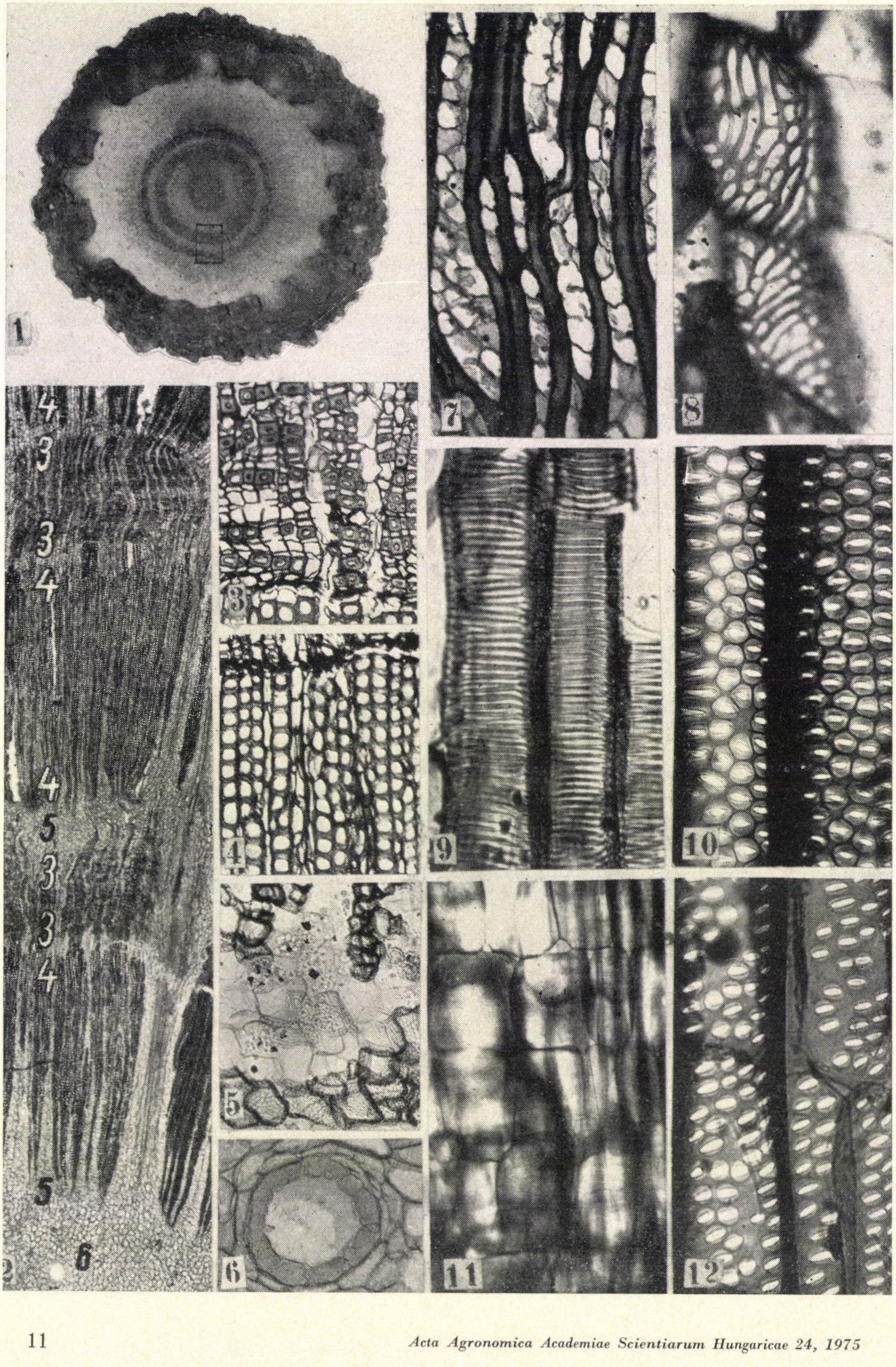
Xylotomic characteristic of Cycads. *Fig. 1.* Cross-section of *Cycas revoluta* stem 1/2 nat. size. *Fig. 2.* Internal structure of conductive bundles (×15). One detail of Photo 1. In Fig. 2. Phloem bundles (3), Xylem bundles (4), Region of transfusion-cells (5). Mucilage ducts (6). *Fig. 3.* Phloem bundles. Cycas media (×100). *Fig. 4.* Xylem bundles *Cycas media* (×100). *Fig. 5.* Transfusion-cells. *(Macrozamia)* (×150). *Fig. 6.* Mucilage duct. *(Stangeria)* (×200). *Fig. 7.* Tangential structure, among the phloem bundles. *(Cycas rumphii).* (×80). *Fig. 8.* Transfusion-cells with reticular thickenings *(Zamia)* (×400). *Fig. 9.* Batten-like thickening of tracheids *(Zamia muricata)* (×400). *Fig. 10.* Araucaroid thickening of tracheids. *(Encephalartos)* (×200). *Fig. 11.* Thin-walled ray cells without thickening. (*Bowenia*) (×200). *Fig. 12.* Cross-field structure of ray cells. The pitting is araucaroid. *(Encephalartos)* (×200).

Plate VII



(x) while inside the Sclerenchyma dorsale the phloem (ph) (Plate III, Fig. 4, Plate VI, Fig. 3b, Plate IX, Fig. 2). The basic tissue consists of parenchyma cells, their walls being more or less thickened. In the walls of fibre tracheids of the ground tissue, there are often silex crystals of peculiar desings, so-called stegmata to be observed (Plate IX, Fig. 3) (st) whose shape and structure change according to the species, in that way having a role in identifying the species.

In the wall structure the vessels can be of wide cavity and gradually thickened or of narrower cavity and spirally thickened (Plate IX, Figs 4, 5, and 6). The perforation of vessels is always scalariform (Plate IX, Figs 7, 8, and 9). The shape of perforation, as well as the thickness and design of the single fibres, change according to the single species and genera. Only Palmae have a structure like that; therefore, they can be well distinguished from other kinds of wood.

IV. *Xylotomic characteristic of dicotyledonous trees* (Plates IV, VI and X). Plate IV shows the three-dimensional perspectivic structure of a two years old linden-branch. The pith is formed by thin walled tissue components to be found in the axis of the young wood body (17). Inside the wood body, in the concentric annual rings, we distinguish earlywood (9, 10) and latewood (11, 12). At the contact of the single annual rings there are the annual ring borders. The single annual rings are interrupted in a radial direction by the rays (14). Between the xylem and phloem components there is the cambium (13). Further there are the cortex (3), then the cortical parenchyma (2) and, at the site of the epidermis, the cork cortex (1). In the sectional structure, the cavities of different size are the cross sections of the leading wood components, of vessels and tracheids. All these details can be well observed in the photograph 4b of Plate VI. The various sectional structures are similarly well presented in column 1 of Plate X where Fig. 1 shows the structure of a wood with ring-pores (Syringa). Fig. 2 is a wood of scattered pores *(Sambucus)*, Fig. 3 is a pore ray *(Corylus)*, Fig. 4 is a pore group (*Laburnum alpinus*), Fig. 5 contains vessels of flamboyant arrangement *(Rhamnus)*.

The photographs of column 2 show the tangential structures of rays. Fig. 6 is a heterogeneous ray skeleton *(Olea europea)*, Fig. 7 is a homogeneous ray skeleton (*Sorbus aria*), Fig. 8 shows mono- and multiseriate rays *(Quercus)*, Fig. 9 shows tyloses in the vessel *(Maclura)*, Fig. 10 is a cumulated ray *(Alnus)*.

In column 3 we see the radial structures of dicotyledonous trees. Fig. 11: Vasicentric parenchyma cells on the vessels (V) *(Diospyros)*, Fig. 12: Paratracheal parenchyma cells in *Quercus* (P). Fig. 13: corner cells on the rims of rays (M) *(Populus)*. Fig. 14: Simple perforation (P) *(Euphorbia)*. Fig. 15: Scalariform perforation and opposed pits (o) *(Liriodendron)*.

(The description and photographs concerning the xylotomy of dicotyledonous trees can be found in detail in P. Greguss's work: "Holzanatomie der europäischen Laubhölzer und Sträucher.) See: References.

*

The most characteristic xylotomic feature of Conifereae. Column. I. Cross sections. *Fig. 1.* The vessels are rounded, without annual ring border. *(Araucaria)* (×200). *Fig. 2.* Cross section with annual ringborder. (a) earlywood, (b) latewood. *(Abies)*. (×100). *Fig. 3.* Resinparenchyma. *(Abies)* (×100). *Fig. 4.* Thin-walled resin duct *(Pinus)* (×100). *Fig. 5.* Thick-walled resin duct *(Picea)* (×60). Column. II. Tangential structure. *Fig. 6.* Uniseriate height rays. *(Abies)* (×100). *Fig. 7.* Rays with resin ducts. *(Pinus)* (×100). *Fig. 8.* Cupressoid thickening of ray cells *(Cupressus)* (×400). *Fig. 9.* Simple pitting in the tangential walls. (*Abies*). (×300). *Fig. 10.* Heterogeneous ray *(Pinus)* (×200). *Fig. 11.* Smooth end-wall *(Taiwania)* (×300), *Fig. 12.* The wall thickened like a necklace of pearls *(Juniperus)* (×300). *Fig. 13.* The wall thickened like a cogwheel. *(Taxodium)* (×300). *Fig. 14.* Very high ray. *(Taxodium)* (×200). Column. 3. Different cross fields of rays. *Fig. 15.* Pinoid pit *(Pinus)* (×300). *Fig. 16. Taxodioid* pit. *(Taxodium)* (×300). *Fig. 17.* Cupressoid pit. *(Cupressus)* (×300). *Fig. 18.* Podocarpoid pit. *(Podocarpus)* (×300). *Fig. 19.* Dacrydioid *(Circoporus)* pit. *(Dacrydium)* (×300).

Plate VIII

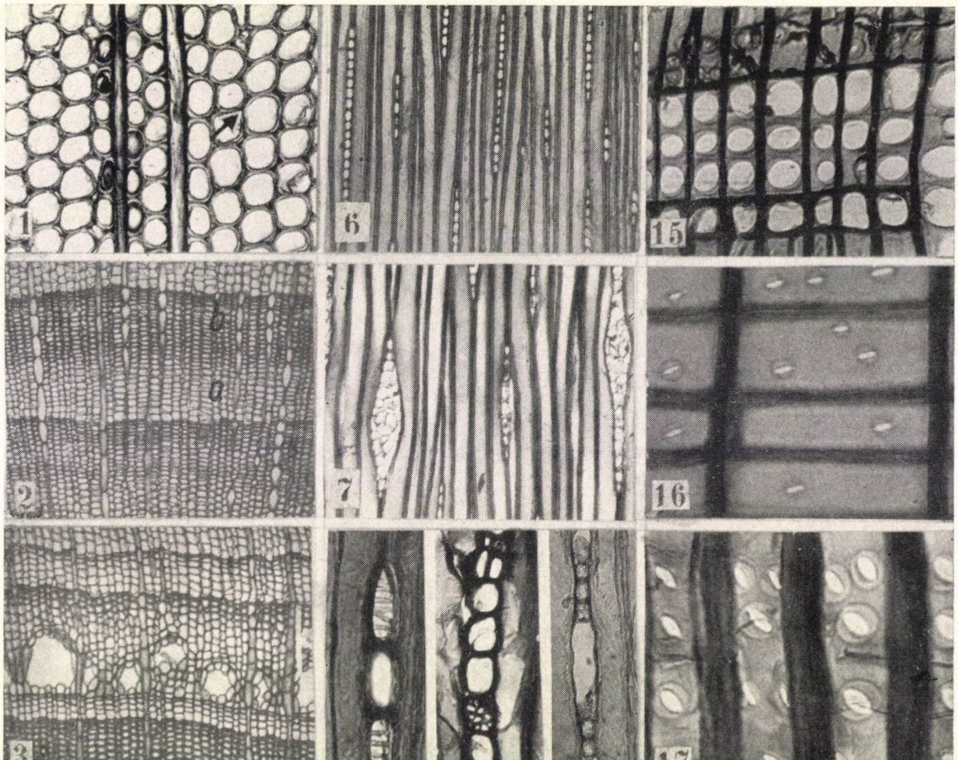
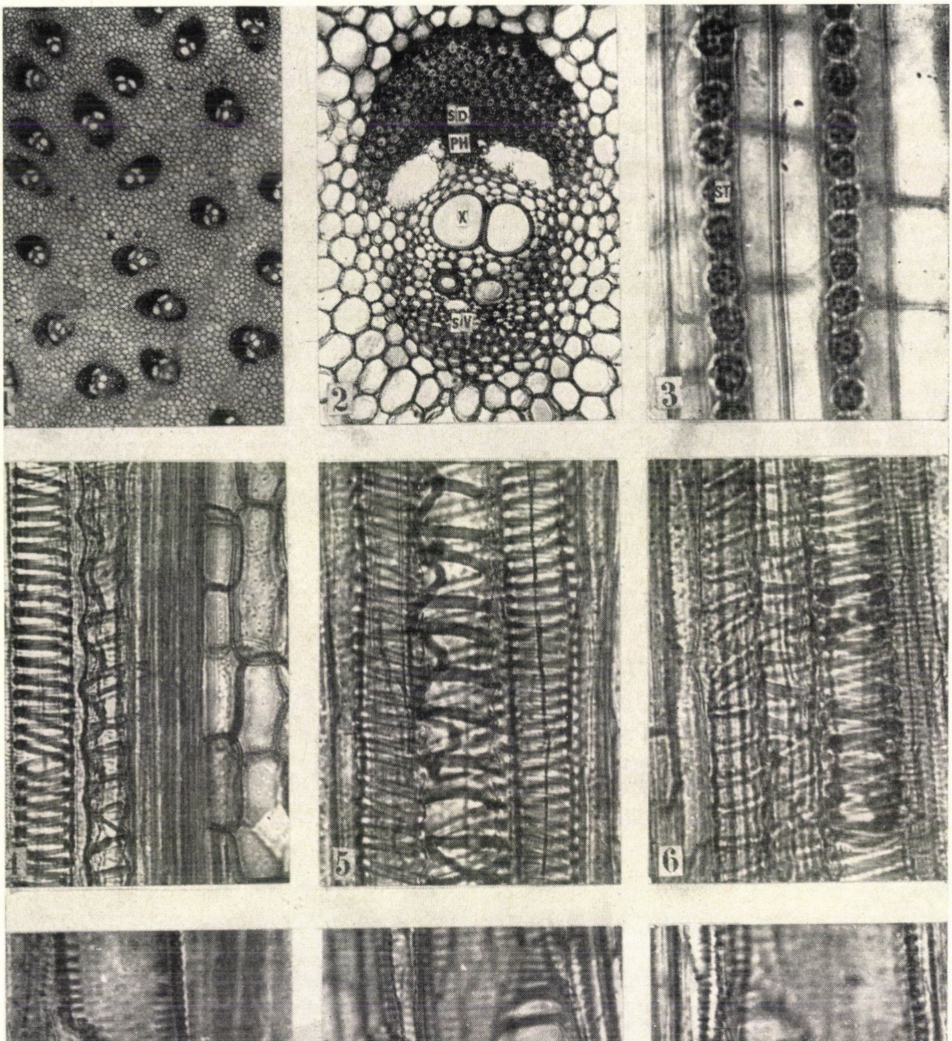


Plate IX



Xylotomic characteristics of monocotyledonous trees *(Palms)*. *Fig. 1.* Among the ground tissue there are single collateral closed vessel-bundles. (*Raphis)* (×30). *Fig. 2.* One single closed vessel-bundle. Sclerenchyma dorsale (SD), Sclerenchyma ventrale (SV), Phloem (PH). Xylem (X), (×200). *Fig. 3.* In the walls of fibre tracheids there are stegmata (ST) (×200). *Fig. 4.* Spiral thickening in the vessels. (×300). *Figs 5—6.* Ring-like thickening in the vessels. (×300). *Figs 7—9.* Scalariform perforations in the vessels (×300).

Apart from the great practical and scientific importance of wood we must not forget the great aesthetic significance of trees, either. A man weary of work but having a recipient mind, in his free time will always look for the free nature, meadows, pastures and the umbrageous forests for being refreshed in body and spirit by their silence and beauty. In these cases, the particular silence of the wood, the play of sunshine glimmering through the foliage of the forest, the twittering and chirping of the birds, and the thousand-faced life and mystery of the forest profoundly affect even people of callous heart. For those non-initiated, the trees of the forest are always dumb but if somebody — as a consequence of the above-discussed facts — had obtained a little insight into the mysterious interior life of trees, he does understand them and in the course of his excursions doesn't mind talking away the time among them. For this very reason it is worth while knowing the inner structure of trees.

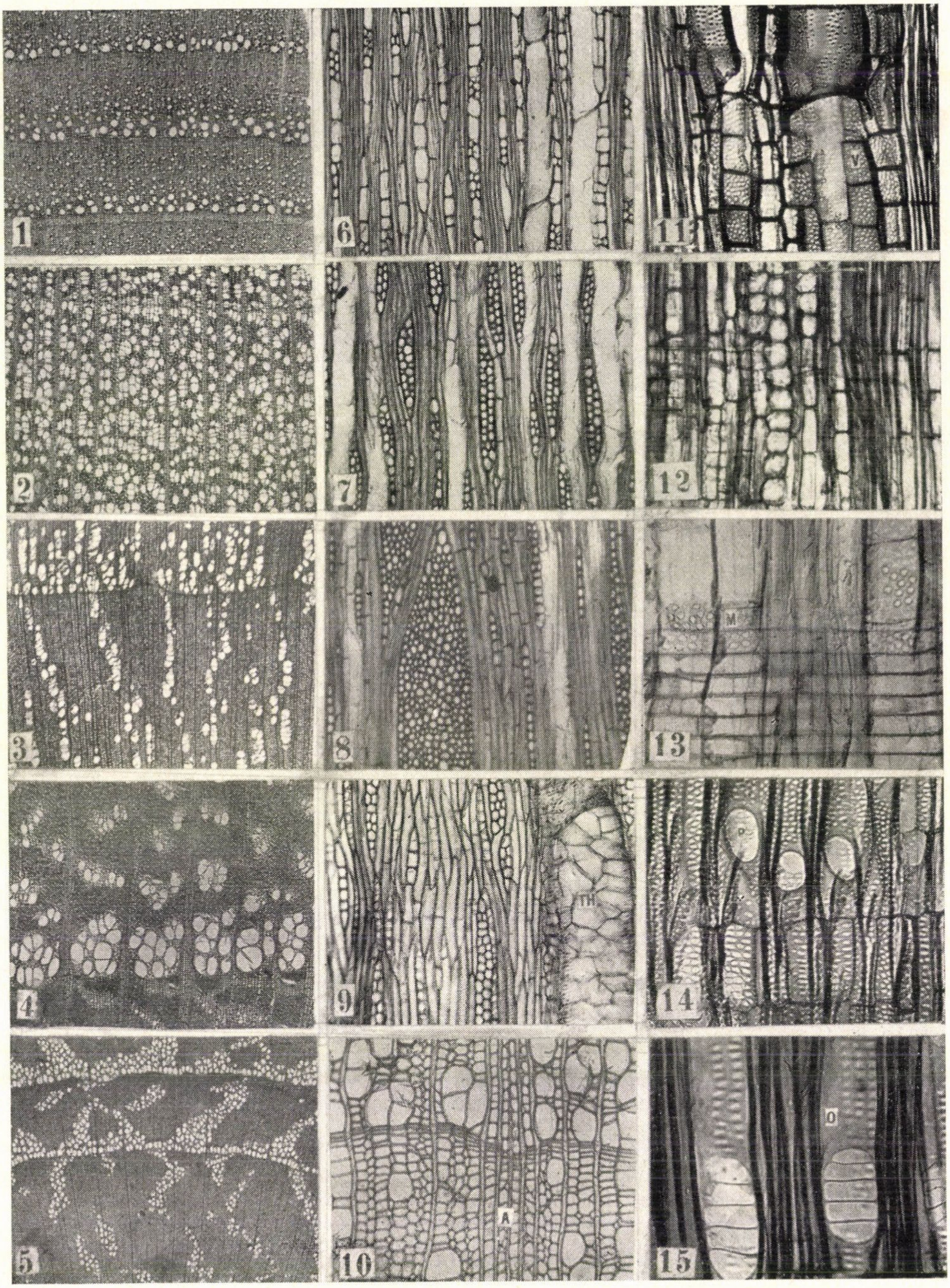
*

Prepared at the Institute for Plant Morphology and Systematic of the Attila József University, Szeged.

P. Greguss

Xylotomic characteristic of dicotyledonous trees. Column. I. Cross sections *Fig. 1.* Showing the structure of a wood with ring-pores. *(Syringa).* (×50). *Fig. 2.* Wood with scattered pores *(Sambucus)* (×50). *Fig. 3.* Pore ray *(Corylus)* (×50). *Fig. 4.* Pore group *(Laburnum alpinum)* (×50). *Fig. 5.* Vessels of flamboyant arrangement *(Rhamnus)* (×50). Column. II. Tangential structures of rays. *Fig. 6.* Heterogenous ray skeleton. *(Olea europea)* (×100). *Fig. 7.* Homogeneous ray skeleton (*Sorbus aria*). (×100). *Fig. 8.* Mono- and multiseriate rays, *(Quercus)* (×100). *Fig. 9.* Tyloses in the vessel *(Maclura)* (×100). *Fig. 10.* Aggregate ray. *(Alnus)* (×100). Column. III. Radial structures of dicotyledonous trees. *Fig. 11.* Vasicentric parenchyma cells on the vessels. (V). *(Diospyros).* (×300). *Fig. 12.* Paratracheal parenchyma cells in *Quercus.* (P). (×300). *Fig. 13* Marginal cells on the rims of rays. (M) (×300). (*Populus*). *Fig. 14.* Simple perforation (P) *(Euphorbia)* (×300). *Fig. 15.* Scalariform perforation (P) and opposed pits (0) *(Liriodendron)* (×300)

Plate X



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THE ANALYSIS OF ADDITIVE AND DOMINANCE GENETIC VARIATION IN A DIALLEL CROSS OF JUTE (CORCHORUS OLITORIUS L.)

The practical utility of diallel crosses in the present day plant and animal breeding is well known. Diallel crosses have been used in predicting the genetic nature of the genes in many crops including lima beans (**Allard** 1956); wheat (**Whitehouse** *et al.* 1958, **Lupton** 1961); pearl millet (**Ahluwalia** *et al.* 1962). Earlier **Jinks**—**Hayman** (1953) developed a method of analysing the diallel table for studying the different genetical situations. **Singh** (1971, 1972) studied the genetical behaviour of the different quantitative characters in the cultivated jute *(C. capsularis)*. However, not much work seems to have been done in this direction. The present study was taken up to have an idea of the additive and dominance effects of genes in a set of diallel crosses in this crop.

A set of 7 × 7 diallel crosses was attempted in *C. clitorius* during 1968 using seven established cultivars i. e. JRO—632, Crumpled leaf, Tobacco leaf, Internode mut, Deep red, Russian red and R—26 (Denoted as P_1 to P_7), in both directions. All the resulting forty nine combinations including the reciprocals were planted in 1969—70 in two replications after proper randomization along with the seven parental selfs. Each plot consisted of three rows each one being 400 cm long and contained more than two hundred plants. The plants were raised with the usual agronomic practices. The data on plant height (cm), basal diameter (cm), number of internodes, size of internodes (cm) dry stick weight (gm) and dry fibre weight (gm) per plant were recorded from twenty randomly selected plants from each plot.

The data were analysed using HAYMAN's (1954) method developed for testing the additive and dominance effects in the diallel crosses. The analysis follows the Latin square general method, the diallel table is superimposed upon the Latin square and each letter indicates a set of single crosses which may be performed apart from the other sets. In the analysis of variance the sum of squares is computed in the same way as in a normal Latin square.

Suppose the measured character is controlled by genes at "K" loci. If the genes are independent in action and show additive behaviour, the measurement in the cross will be the mean of the two parental measurements only. Maternal effects may sometimes be present and in that case the means of the reciprocal crosses are also taken into account.

Let Y_{rs} be the entry in the rth row and sth coloumn in the diallel table. The additive variance between the parents and the maternal effects in the cross may be tested as:

$$Y_{rs} = m + j_r + j_s + j_{rs} + k_r - k_s + k_{rs}$$

Where m = grand mean j_r = mean, deviation from the grand mean due to the rth parent, j_{rs} = remaining discrepancy in the rsth reciprocal sums, 2 k_r = difference between the effects of the rth parental line used as male parent and as a female parent, 2 k_{rs} = remaining discrepancy in the rsth reciprocal difference.

The four sum of squares are denoted as (a), (b), (c) and (d) and measure the above four variations. This analysis was however, given by YATES (1947).

The deviations of the progeny from their parental mean depend on the dominance and the (b) mentioned above measures this variation. The components (a) and (b) may further be interpreted more precisely through some other biometrical genetical models; MATHER (1949) developed such models for studying the polygenic systems. If the number of parents used in the crosses are usually more than two, a polygenic system is considered (for details see HAYMAN 1954).

After extending the linear statistical model, the constants for the dominance difference between parental mean, progeny mean, and the deviations due to specific parents, may be fitted. The component (b) will then form a new corresponding sum of squares and this can be explained genetically as:

$$\begin{aligned} Y_{rs} &= m + j_r + j_s + l + l_r + l_s + l_{rs} + k_r - k_s + k_{rs} (r \neq s) \\ Y_r &= m + 2j_r - (n - i)\, l - (n-2)\, l_r \end{aligned}$$

The new constants are

l = mean dominance variation
l_r = further dominance deviation due to the rth parent
l_{rs} = remaining discrepancy in the rsth reciprocal sum.

Therefore, in terms of the biometrical genetical model the mean square b_1 will be

$$= n^2 \sum_i \sum_{ab} u_{ai}\, u_{bi}\, h_{abi}\, (^2)\, (n-1) + \sigma_e^2$$

and $b_2 = 4n \sum_i \sum_a u_{ai} (\sum_b u_{bi}\, h_{abi} - \sum_{bc} u_{bi}\, u_{ci}\, h_{bci}\, (^2)\, (n-2) + \sigma_e^2$

b_3 also estimates dominance.

Each error entered in Table 1 is the interaction with the environment of the corresponding mean effect and since the additive and dominance variation may not be expected to be influenced to the same extent by the environment, each mean should be tested against its own interaction. However, the error variances have been pooled to give a common error variance (Bt) and the mean in each case has been calculated by using it.

Table 1

Analysis of variance for different quantitative characters in jute

Components	Constants	df	Mean sum of squares for different characters					
			plant height	basal diameter	number of internodes	size of internodes	stick weight	fibre weight
a	j_r	6	1152**	0.015	42.33**	0.03**	393.09**	47.39**
b1	l	1	21918**	0.031	0.22	0.39**	2385.31**	688.48**
b2	l_r	6	9360**	0.101**	98.83**	0.06**	167.97**	41.14**
b3	l_{rs}	14	1058**	0.042	113.41**	0.11**	523.60**	88.32**
b	j_{rs}	21	4423**	0.059**	103.86**	0.11**	505.88**	103.47**
c	k_r	6	1152*	0.015	17.00	0.02*	51.19*	2.92
d	k_{rs}	15	1071*	0.080	45.20*	0.02*	12.90	3.76
		48	2558	0.054	48.56	0.06	255.09	42.78
B		1	10595	0.330*	4.00	0.01	64.22**	8.70
Ba		6	36357**	7.310**	1650.50**	9.36**	5218.89**	990.75**
Bc		6	148	0.0005	24.50*	0.03	19.83	5.83
Bt		48	998	0.034	18.33	0.002	24.45	9.75

** = $P < 0.001$
* = $P < 0.05-.01$

1) Plant height. All the components were tested for their significance. It may be seen from Table 1 that (a), (b), (c) and (d) were significant. The significance of (a) indicated that the cultivars used show genetical variation in them, the significance of (b), which measures the reciprocal differences, showed that types had dominance for this character at some of the loci. The components (c) and (d) were also found to be significant to some extent. This indicated that the types depict some maternal effects in the crosses for plant height. However, the variation present in the reciprocal crosses may not be said to be due to maternal effects.

Regarding the dominance genetic variation, its various components (b_1), (b_2) and (b_3) showed significance. The significance of (b_1) clearly indicated the presence of dominance for this character. The significance of (b_2), however, showed a possibility of asymmetrical gene distribution for plant height in the types.

Block differences showed some significance. This indicated that the height had also been affected by the blocks to some extent.

2) Basal diameter. The component (a) did not show any significance for this character. This indicated that the types used here do not show any genetical variation amongst them for basal diameter. The component (b) was significant. As it is known, it measured the reciprocal difference and had a dominance nature of genes at some loci. However, there was no maternal effect because the components (c) and (d) did not show any significance. The significance of (b_2) indicated the asymmetry in the gene distribution for basal diameter in the varieties used here. However, (b_1) did not show any significance, hence it may be said that there is no dominance present.

However, there were block differences. This indicated that this character had been affected to some extent by the blocks also.

3) Number of internodes. The components (a) and (b) showed significance. This indicated that the types show genetical variation among them and also the dominance variation at some loci. However, there was no maternal effect present, hence it may be said that whatever differences were present in the reciprocal crosses, they were not due to the presence of any maternal effect, but may be due to certain other reasons which are difficult to determine at this stage. The component (b_1) was not significant. It may be said that the types lacked dominance effect for this character. The component (b_2) and (b_3) showed significance, indicating the asymmetrical gene distribution for this character.

The blocks also had an effect on the expression of this character.

4) Size of internodes. The component (a) which measures the variation between the mean effects of each parental line, was found to be significant. This indicated that the types used here depict the genetical variation among them, the component (b) was also significant which indicated the dominant nature of genes at some loci. For the size of internodes there seems to be a maternal effect existing, as both (c) and (d) components were found to be significant.

The significance of (b_1) (b_2) etc. indicated the presence of dominance and also the distribution of genes in a most asymmetrical manner in the types.

Block differences existed to some extent.

5) Stick weight. The dry weight of the stick is an important character in this crop after fibre. This character gives an idea of the cambial activity in the formation of ultimate fibre in the plant along with the wood. The component (a) was found to be significant, showing a genetical variation in the types for this character. The component (b) was also significant. It indicated the dominance nature of genes at some of the loci. The component (c) was also significant to some extent which indicated the presence of the maternal effect on this character.

The sub-components of (b) i. e. (b_1) and (b_2) were also significant, this all indicated the presence of dominance and the asymmetrical gene distribution in the types.

To some extent, the block differences also existed.

6) Fibre weight. The ultimate fibre weight per plant gives an idea of the fibre yielding capacity of the cambium. The component (a) was significant, this indicated the presence of genetical variation in the types for this character. The component (b) was also significant showing the presence of the dominance effect of genes at some loci. However, there was no maternal effect present.

The (b_1) and (b_2) were also significant, indicating the presence of dominance and the irregular distribution of genes at some loci.

Block difference for genetical variation in the types was, however, present.

The six quantitative characters which have been studied in this experiment form the ultimate basis of the fibre yield in jute. Plant height, basal diameter, dry stick weight and dry fibre weight had a high correlation with the fibre yield and also form a good criteria of selection in this crop (SHUKLA—SINGH 1967, SINGH 1971b).

The varieties used here showed a genetical variation for plant height, number of internodes, size of internodes, stick weight and fibre weight. It was only in case of basal diameter that no genetical difference was observed among the types. This suggests that the types selected for this study do show genetical divergence of these characters and may yield good results if they are used in a mass breeding programme.

Genetical and geographical divergence if established in crops, provide immense opportunities to workers to improve the types. In this experiment, the varieties collected from different geographical regions were included, along with some of the established mutants. Probably this is one of the reasons why good genetical differences have been obtained.

Variations in the reciprocal crosses which were not due to the variations between the mean effects of each parental line were observed in case of all the characters studied. One should be quite cautious in using these types in the crosses for getting better results. The parent giving better results as a mother should only be used as mother in such crosses.

Maternal effects were, however, observed in characters like plant height, size of internodes, stick weight and fibre weight.

The dominance genetic variation was observed through the components like (b) and (b_3). They give an idea of the mean dominance deviation, dominance deviation due to the particular parent and remaining discrepancy in the rsth reciprocal sums, respectively. The presence of dominance genetic variation was observed in case of plant height, size of internodes, stick weight and fibre weight. Almost all the characters showed asymmetrical gene distribution (b_2). The asymmetrical gene distribution may help us in the proper exploitation of these characters and ultimately getting to the goal of improving fibre quantity in this crop.

The differences due to blocks were also present to some extent. This was due to the fact that one of the blocks was partly affected by incessant rains, just a month before the final harvest.

The analysis of the diallel table by this method gives a rapid idea of the genetic variation ascribable to the additive and dominance causes.

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*

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CHEMICAL COMPOSITION AND NUTRITIVE VALUE OF EGYPTIAN TOMATO

Tomato *Lycopersicum esculentum*, Balady variety is the most widely cultivated vegetable fruit in Egypt. There is lack of sufficient data concerning its chemical composition, especially the type of sugars and amino acid components. El-Tinay (1972) showed that different tomato varieties differed somewhat in their chemical composition. This difference was most pronounced in their total solids, minerals, and the lycopene content. There was only slight difference in acidity. McCance—Widdowson (1960) in their report gave the chemical composition of the tomato. The present work was carried out to study the chemical composition and nutritive value of Egyptian tomato with main emphasis on the sugars and the free amino acids of the fruits.

Sampling. Five kg. of fresh tomato fruits, Balady variety collected from Giza province neighbourhood were used in this study. The fruits were blended and the juice was extracted and analysed.

Analytical methods. The juice was analysed for its moisture, ash, protein, fat, total soluble solids, iron and acidity using the usual standard methods (A. O. A. C. 1970). Calcium and phosphorus were estimated using Stuffins method (1967). Caroteinoids were determined by the method given by Wettestein (1957) and lycopene by Wong—Bohart (1957). Ascorbic acid was determined by the 2.6-dichlorophenol indophenol titration method (Leonard—Marsh 1958).

The tomato juice was filtered after centrifugation. Sugars and amino acids of the tomato filtrate were identified and quantitatively estimated as was previously and quantitatively estimated as was previously done by Salem—Hegazi (1973).

The results obtained in the present study are summarized in the following tables. Table 1 shows the general chemical composition of tomato fruits. It can be shown that monosaccharids represented by glucose and fructose were present in appreciable amounts. Meanwhile, the data concerning the carotenoids, ascorbic acid, lycopene, glucose, phosphorus, calcium and acidity were in the range previously reported (El-Tinay 1972). These findings show that tomato may be considered as a source, not only for sugars and proteins but also as a valuable source for carotenoids and ascorbic acid, besides its high calcium and phosphorus contents.

Table 2 shows the daily requirements of the main important nutritional elements present in tomatoes. From the table it can be seen that tomatoes can be considered as an excellent and cheap source of vitamin C. It is also a relatively cheap source of vitamin A as it contains about 600 I. U. of it. It contains quite a fair amount of important nutritional minerals, mainly calcium, phosphorus and iron.

The free amino acis pattern in the tomato fruit is illustrated in Table 3. These results give valuable information about the amino acid constituent in the Egyptian tomato fruits, since no available data have been published about the nature and type of amino acids. It can

Table 1

The chemical composition of the Egyptian tomato fruits (Balady variety), (Dry basis)

Chemical constituents	Tomato fruits
Moisture, %	94.00
Fructose, %	28.88
Glucose, %	32.72
Total protein nitrogen, %	16.00
Fat (ether extract)	Traces
Crude fibre, %	21.40
Total ash, %	9.60
Calcium (mg/100 g)	100
Phosphorus (mg/100 g)	300
Iron (mg/100 g)	9
Carotenoids (mg/100 g)	58
Lycopene (mg/100 g)	125
Ascorbic acid (mg/100 g)	364
Titrable acidity % (as citric acid)	9.28
pH of the juice	4.3

Table 2

The vitamin and mineral content of tomato as compared with the daily requirement for reference man

Item	Tomato contents (mg/100 g) (Wet basis)	Daily requirement for reference man
Vitamin C	21.84	30 mg
Carotene	3.48 = 580 I.U. vit. A	5000 I.U.
Calcium	6	800 mg
Phosphorus	18	800
Iron	0.58	12 mg

be seen that fifteen amino acids were detected and quantitatively determined. Lysine, histidine and arginine are present in higher levels than other amino acids. Glutamic and aspartic acids are present in larger amounts in the juice of tomato fruits, while proline showed the least value of amino acid found.

*

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Table 3

*Free amino acid content of the Egyptian tomato juice**
(Dry basis)

Amino acids	mg/100 g
Aspartic acid	125
Glutamic acid	155
Serine	100
Glycine	75
Threonine	42
Alanine	86
Valine	35
Leucine isoleucine	82
Phenylalanine	66
Lysine	350
Arginine	205
Histidine	290
Methionine	35
Proline	15

* Tomato juice represents 88% of the fruits.

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PLAST STRUCTURE OF VARIOUS SHOOTS OF EQUISETUM ARVENSE L.

The fine structure of the chloroplast having been clarified (Granick 1961, Menke 1964) and the leucoplasts studied (Mikulska 1960, Lichtenthaler—Peveling 1967) plasts equally performing assimilating and storing functions too were detected (Ledbetter—Saleme 1967).

The present paper gives the detailed results of investigations on *Equisetum arvense* shoots performing three different functions. From the rhizome of the perennial *Equisetum* first a brown fertile shoot of heterotrophic nutrition producing spores develops in spring, then somewhat later, at the end of May, a green verticillate autotrophic sterile shoot is formed. Since the light microscope studies revealed differences between the shoots in the structure of the primary cortex under the epidermis, we subjected this tissue zone to electron microscope examinations and studied the structural characteristics and differences of the plasts.

The test material taken from three different shoots was fixed in a 2 per cent buffer treated potassium permanganate solution, then embedded in araldite. Contrasting was carried out with uranyl acetate and lead citrate.

We begin our discussion with the fine structure of the green sterile shoot. In the chlorenchyma of the primary cortex the cytoplasm of the cells shows a fine network, beside the chloroplasts along the cell-wall small proplasts and mitochondria are found (Fig. 1). In the granular chloroplasts of highly differentiated structure the stroma-thyllakoids run down in sharp curves (Fig. 2). Toward the end of the assimilation, in the afternoon hours, starch grains appear pushing the stroma lamellae aside.

In the primary cortex of the yellowish brown fertile shoot the cell nucleus has a central position. Various size vacuoles divide the cytoplasm into two parts: one surrounding the nucleus and the other located along the cell-wall; the two parts are connected by plasm-bridges. The plasts are mostly formed round the nucleus and only get closer to the cell-wall through the wider plasm-bridges (Fig. 3). Here the internal membrane system of the plasts has but slightly developed. However, beside the thyllakoids pressed to one side of the plasts one or more rather large starch grains have been formed (Fig. 4). Thus, as regards their fine structure these plasts represent an intergrade, the so called chloro-amyloplast.

As a comparison we examined the structure of the primary cortex in an underground organ: the rhizome. Here round the large central vacuole, in the specially formed cytoplasm along the cell-wall large leucoplasts were found with well developed starch grains (Figs 5, 6). As to their fine structure, the leucoplasts are perfectly without stroma and grana lamella.

Summing up, as regards the function and structure, the three different shoots of *Equisetum arvense* contain three different types of plast. We think it necessary to emphasize that the difference between the plasts of sterile and fertile shoots is not simply one of the development stage. In this case we have to suppose certain biochemical, or possibly even genetic factors required for the pigment formation of normal chloroplasts and complete differentiation of the internal membrane system to be missing from the so called chloro-amyloplasts of the fertile shoot.

*

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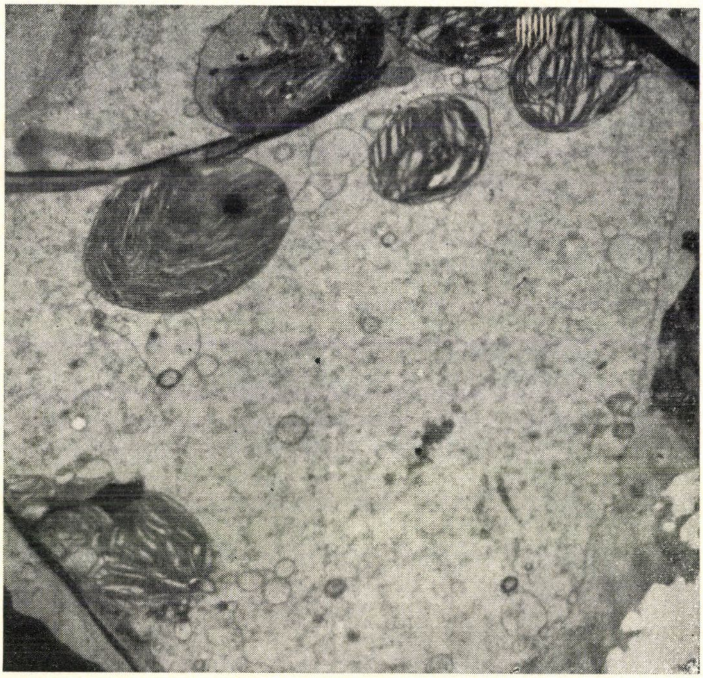

Fig. 1. Part of a green sterile shoot of *Equisetum arvense*, with chloroplasts. (2800 ×)

Fig. 2. Chloroplasts with sharply winding stromathyllakoids. (15,000 ×)

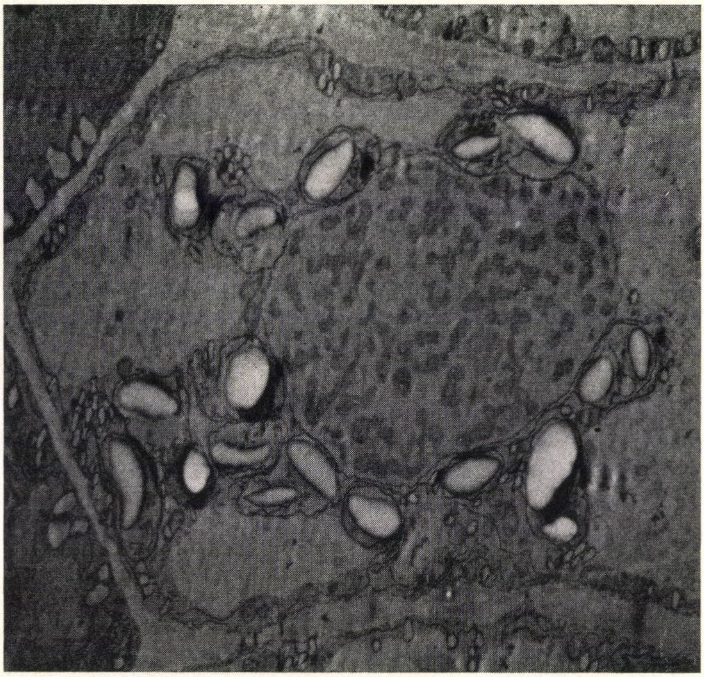

Fig. 3. Part of a brown fertile shoot of *Equisetum arvense*, with chloro-amyloplasts round the nucleus. (2800 ×)

Fig. 4. Part of the former, with starch grains in the chloro-amyloplasts round the nucleus. (15,000 ×)

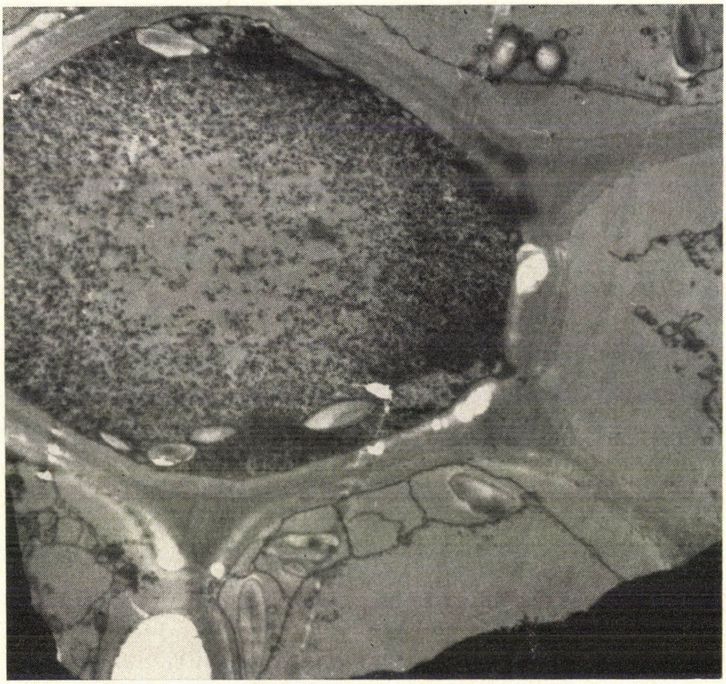

Fig. 5. Part of the rhizome of *Equisetum arvense.* (2800 ×)

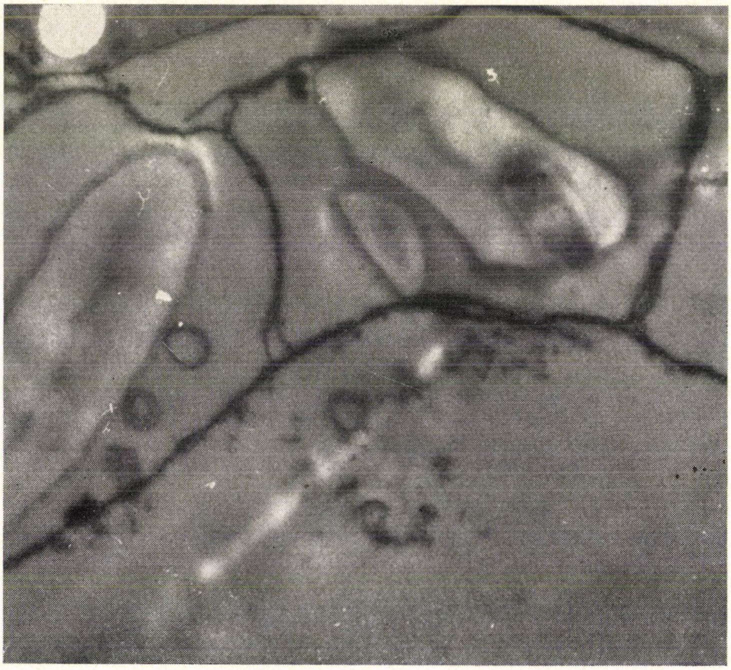

Fig. 6. Part of the former, with starch grains stored in the leucoplasts. (15,000 ×)

SEROLOGICAL STUDIES ON THE TOMATO STREAK STRAIN OF TOBACCO MOSAIC VIRUS

Serological techniques appear to be of value in the study of plant viruses. Using the rabbit as an experimental animal, Mulvania (1926) and Birkeland (1934), Enrique Perez—Adsuar (1954), Alvarez—Accatino (1962) prepared specific antibodies of tobacco mosaic virus (R/1 : 2/5 : E/E : S/ *) and sugarcane mosaic virus (*/ * : */ * : */ * : S/Ap). Chester (1936) sensitized guinea pigs with tobacco mosaic virus.

Newton—Edwards (1936) produced chicken antiserum from *Datura* plants infected with potato virus X R/1 : */6 : E/E : S/Fu).

Beale—Lojkin (1937), D'yachenko—Khrutskil (1958—1959) prepared tobacco mosaic virus antiserum from horses.

In this research serological studies were carried out on the tomato streak strain of tobacco mosaic virus.* These studies were made to elaborate an antiserum against the virus into rabbits and chickens and to determine the conditions for maximum antibody formation through changing the amount of purified virus injected intravenously into chickens or rabbits.

Tobacco being of international economic importance was chosen for these studies on the virus.

To elaborate an antiserum against the tomato streak strain of tobacco mosaic virus in chickens and in rabbits the following procedure was followed:

1. Purification of the tomato streak strain of tobacco mosaic virus from infected *Nicotiana tabacum* L. var. *angustifolia* plants.
2. Determination of the dilution end point of the different fractions obtained during the purification processes of the virus by infectivity tests on *Nicotiana tabacum* L. var. *angustifolia*.
3. Immunization of chickens and rabbits against different doses of purified virus preparations.
4. Precipitation tests with immune sera of chickens and rabbits against heat-clarified virus preparation at different dilutions.

Purification of virus. A stock solution of purified virus was prepared as follows:

1. Three hundred two-month-old seedlings of *Nicotiana tabacum* L. var. *angustifolia*, were inoculated with the tomato streak strain of tobacco mosaic virus. The infected sap was applied by a brush to the leaves dusted with 600-mesh carborandum, as recommended by Rawlins—Tompkins (1936). The inoculated leaves were washed quickly with tap water. After 20 days mosaic symptoms appeared very clearly on the non-inoculated leaves.

The infected tobacco leaves were collected, washed with distilled water and frozen for 24 hours. 600 ml of juice was expressed by grinding the frozen leaves with a pestle and mortar, and the sap was passed through a piece of cheesecloth.

2. 500 ml of expressed infective sap from frozen tissue was heated at 55°C for 10 minutes to coagulate plant residues.
3. The supernatant was centrifuged at 3000 rpm for 10 minutes to throw down heavy coagulum.
4. The clarified sap was ultracentrifuged in a Spinco Model L Centrifuge at 144,000 g (40,000 rpm) for 60 minutes using 132 ml of the sap each time.
5. The supernatant was tested for the presence of the virus. The remaining pellets at the bottom of the tube were gelatinous, 1 cm in diameter, not easily soluble in water, but were crushed with a glass rod. They were suspended in 13.2 ml of sterile distilled water. Thus the

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* Eskarous—Habib (1970) identified a severe disease of tomatoes (*Lycopersicum esculentum* Mill. cultivar "Marmande") as tomato streak, caused by a single virus which is probably due to a strain of tobacco mosaic virus.

virus suspension was $132 = 10$ times as concentrated as the original suspension. A trial to add 0.2 M phosphate buffer solution at pH 7.0 caused a white precipitate.

6. The suspension of the virus in water was again centrifuged at 2000 rpm for 10 minutes and the supernatant which is the purified virus suspension was kept in ampoules in a freezer.

7. The final purified fraction was examined under an electron microscope. Virus magnification was 48,000 times.

Infectivity of the different fractions obtained during the purification process. For a determination of the infectivity of purification fractions and purified virus preparations on *Nicotiana tabacum* L. var. *angustifolia*, the following dilution end points were determined:

a) Dilution end point of untreated sap from infected tobacco leaves.

b) Dilution end point of sap heated for 10 minutes, at 55°C.

c) Dilution end point of sap heated and centrifuged at 3000 rpm termed heat-clarified infected sap.

d) Dilution end point of remaining pellets after ultra-centrifugation diluted to the original volume.

e) Dilution end point of supernatant obtained from ultracentrifugation process at 40,000 rpm.

f) Dilution end point of ultracentrifugated sap after its centrifuging at 2000 rpm.

For a determination of the dilution end point of the infected sap at each step of purification, the sap was diluted in tenfold dilutions starting from 10^{-1} to 10^{-8}. The same process was repeated using dilutions between the two final dilutions of each fraction. Each dilution was inoculated into a number of healthy *Nicotiana tabacum* L. var. *angustifolia* plants. The number of plants showing infection was determined and the percentage calculated.

Preparation of the antiserum in chicken. An antiserum was prepared by successive intravenous injections in chickens with the above purified tomato streak strain of tobacco mosaic virus.

1 ml of the purified virus preparation, 10 times as concentrated as the original virus suspension was injected intravenously into each of 3 chickens variety Nechols each weighing about 1.75 kg. The injection was made into the veins which run along the under surface of the wing. The small feathers were plucked, and the wing was wiped with a piece of cotton dipped in alcohol. The tip of a 2 ml syringe with a thin size 16 needle was inserted into the vein in the direction of the base of the wing. Six injections were carried out at 5 day intervals.

A test bleeding was made most commonly 5 days after each injection by extracting 2.5 ml of blood by heart puncture. The blood was allowed to clot at 4°C for 2—3 hours. The separated serum was centrifuged at 3000 rpm for 10 minutes, by which clear antiserum was obtained. The serum was kept in a refrigerator after the addition of a few drops of chloroform. Successive five bleedings at 5 day intervals, and another three bleedings at 4 day intervals after the last injection were carried out.

The antiserum was tested against heat clarified virus-containing sap diluted 1 : 10 and 1 : 20.

In rabbits. 3 rabbits variety Polish of about 2 kg each were used for preparing the antiserum against the above purified tomato streak virus. They had fairly large ears with prominent veins.

1 ml of the purified virus preparation, 10 times as concentrated as the original virus suspension was injected intravenously into the rabbit. The injection was carried out as described by SMITH (1960). The injection was repeated 6 times at 5 day intervals within 25 days. The first injection was given near the tip of the ear, each successive injection closer to the base. 5 days after the 1st injection, the rabbit was bled from the other ear. Before bleeding, the ear was rubbed with a small piece of cotton dipped in xylene. A small cut was then made in the marginal vein near the base of the ear, using a small very sharp scalpel. After taking 2 ml

of blood, the bleeding was stopped by applying slight pressure, and the cut sealed by a small quantity of collodion dissolved in alcohol-ether.

A test bleeding was made 5 days after each injection. 5 bleedings were carried out. Within the 12 days after the last injection, 3 more bleedings were carried out at 4 day intervals. The blood was collected in a tube and left to clot for some hours at 4°C. The separated serum was then centrifuged at 3000 rpm for 10 minutes. The serum was tested against heat clarified virus-containing sap diluted 1 : 10 and 1 : 20.

Precipitation tests on chicken serum. The precipitation reaction between the tomato streak strain of tobacco mosaic virus and the serum produced after each bleeding was carried out.

Two sets each containing a series of two-fold dilutions of the antiserum in 85% sodium chloride solution were prepared starting from 1: 2 up to 1 : 1024. An equal volume of heat-clarified infected sap of the tomato streak strain of tobacco mosaic virus diluted 1 : 10 and 1 : 20 was added to each set.

Four control mixtures were prepared: The first consisted of a set of tubes containing serially diluted serum from chicken injected with the healthy purified sap of tobacco leaves plus heat clarified infected sap of tobacco leaves diluted 1 : 20 and 1 : 10. The second contained serum from a chicken which had not been injected with any antigen plus heat clarified infected sap of tobacco leaves diluted 1 : 10 and 1 : 20. The third contained serum from a chicken which had not been injected with any antigen, plus heat clarified healthy sap of tobacco leaves diluted 1 : 10 and 1 : 20. The fourth control contained antiserum plus heat-clarified healthy tobacco sap diluted 1 : 10 and 1 : 20. The contents of each tube of the above sets were mixed by brief shaking. The lower half of the contents of the tubes was immersed in a water bath adjusted at 50—55°C and the tubes were kept for one hour.

Arbitrary units were given to designate the degree of precipitation as follows: ++++ very high precipitation, +++ high precipitation, ++ moderate precipitation, + slight precipitation, ± trace of precipitation, and — no precipitation.

Precipitation tests on rabbit serum. The same procedure as above was followed in the case of rabbit serum.

A trial to increase the amount of virus injected into chickens and rabbits was made. Two ml of purified virus preparation 10 times as concentrated as the original suspension was injected intravenously into chickens and rabbits using the same above-mentioned technique except for increasing the amount of inoculum to 2 ml instead of 1 ml each time.

Purification of virus. Electron micrographs of the purified preparation of virus diluted 1 : 20 and magnified 48,000 times, showed that virus particles appear in the form of needles measuring approximately 156—313 millimicrons in length.

Infectivity of the different fractions obtained during the purification process. Plant under test: *Nicotiana tabacum* L. var. *angustifolia* (Table 1 and Fig. 1).

The untreated infected sap containing tomato streak virus can retain its infectivity at dilutions up to 1 : 10,400,000.

The infectivity of the heated sap is slightly decreased. The dilution end point is 1 : 2,200,000.

The heated centrifuged sap is slightly less infective than the uncentrifuged heated sap. The dilution end point of the heated centrifuged sap is 1 : 2,200,000.

The dilution end point of the purified ultracentrifuged sap is 1 : 1,200,000, indicating that there is no great loss of the virus as only a small amount of virus particles is still suspended in the supernatant solution. The infectivity of this supernatant is very low having a dilution end point of 1 : 1,400.

The dilution end point of the ultracentrifugated fraction remains constant after simple centrifigation (Table 1).

In the figure the percentage of plants showing infection is plotted against dilution.

Precipitation tests on chicken serum injection of the purified tomato streak virus into the blood stream of a chicken resulted in the production of specific antiserum.

The antibody optimum was obtained 5 days after the 4th injection; in other words 20 days after the 1st injection. The reactive antiserum gave titres up to 1 : 64 when treated against virus-containing sap at dilutions of 1 : 10 and 1 : 20. The serum end titre remained nearly constant for 9 days when it began to decrease gradually.

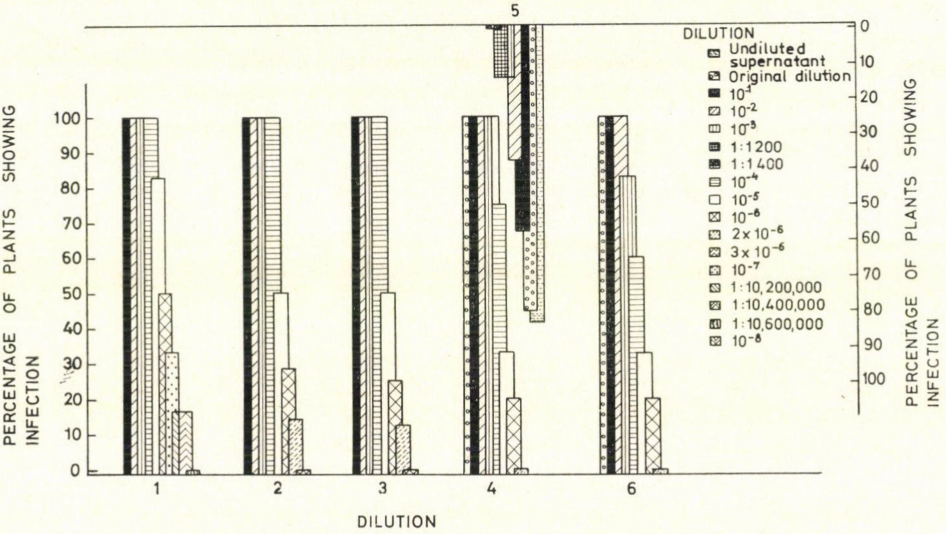

Fig. 1. Dilution end point of different fractions obtained during the purification process of the tomato streak strain of tobacco mosaic virus (1 = Untreated infected sap, 2 = Infected heated sap, 3 = Heated centrifugated sap, 4 = Ultracentrifugated sap, 5 = Supernatant of ultracentrifugated sap, 6 = Ultracentrifugated fraction after simple centrifugation)

The results of the precipitation tests are summarized in Table 2.

The first control which contained serum from chicken injected with healthy purified sap showed a slight precipitate up to 1 : 16 when tobacco sap diluted 1 : 10 and 1 : 20 was added. The three other controls showed negative results.

Precipitation tests on rabbit serum. The injection of the purified tomato streak strain of tobacco mosaic virus into the blood stream of a rabbit resulted in the production of a specific antiserum. The antibody optimum was obtained 5 days after the 4th injection, in other words 20 days after the 1st injection. The reactive antiserum gave titres up to 1 : 512 when tested against virus-containing sap diluted 1:10. The serum end-titre remained constant for 13 days then it began to decrease.

The results of precipitation tests are summarized in Table 3.

The specific precipitates obtained with this group of viruses were of the rapidly forming open flocculent 'H' type commonly produced by rod-shaped viruses such as those of TMV.

All controls gave negative results

Increasing the amount of virus injected into chickens did not greatly alter the antibody optimum or the serum end titre. Results are summarized in Table 4.

Increasing the amount of virus injected into rabbits led to an appreciable increment of the antibody optimum and serum end titre. Results are summarized in Table 5.

The serum end titres of chickens and rabbits injected with 1 ml and 2 ml of purified tomato streak virus are represented in Fig. 2.

Table 1

Dilution end-point of ultracentrifuged fraction after simple centrifugation
Tested plant: Nicotiana tabacum L. var. angustifolia

Dilution	No. of plants inoculated	No. of plants showing symptom	% of plants showing infection
Supernatant diluted to the original dilution	6	6	100
10^{-1}	5	5	100
10^{-2}	6	6	100
10^{-3}	6	5	83.33
10^{-4}	5	3	60
10^{-5}	6	2	33.33
10^{-6}	5	1	20.00
10^{-7}	6	—	0
10^{-8}	7	—	0
2×10^{-6}	6	—	0
3×10^{-6}	6	—	0
4×10^{-6}	5	—	0
1 : 1,200,000	8	—	0
1 : 1,400,000	7	—	0
1 : 1,600,000	7	—	0
1 : 1,800,000	7	—	0

It was noticed that the serum end-titre in case of rabbits was usually higher than that in chickens.

The intravenous injection of the purified tomato streak strain of tobacco mosaic virus into the blood stream of a chicken or rabbit resulted in the production of a specific antiserum. The antiserum reacted positively with the heat-clarified infected sap diluted 1 : 10 and 1 : 20. These results are in agreement with those of ALVAREZ—ACCATINO (1962) who prepared an antiserum against *Nicotiana* virus 1 by the intravenous injection of rabbits with purified virus from diseased tobacco plants.

NEWTON—EDWARDS (1936) used chickens instead of rabbits for the preparation of an antiserum against potato virus X obtained from the sap of infected *Datura meteloides* L. and *Datura stramonium* L. plants.

In our studies it was found that the virus antigen of the tomato streak strain of tobacco mosaic virus is highly precipitogenic. On the other hand the healthy tobacco proteins have a very low order of antigenicity when injected into chickens but nearly no antigenic properties in the case of rabbits. This indicates that the virus is nearly completely freed from the antigenic constituents of normal plants by the use of the ultracentrifuge method of purification. This method at the same time caused no great loss of the virus, as indicated by the infectivity tests carried out for each fraction.

Table 2

Precipitation tests with antiserum from chicken injected with 1 ml of purified sap concentrated 10 times as much as original dilution.
Tests carried out at 5-day intervals with heat clarified sap diluted (1 : 10) and (1 : 20)

Bleeding after (days)	Dilution of virus in saline	Dilution of antiserum and degree of precipitation									
		1 : 2	1 : 4	1 : 8	1 : 16	1 : 32	1 : 64	1 : 128	1 : 256	1 : 512	1 : 1024
5	1 : 10	—	—	—	—	—	—	—	—	—	—
	1 : 20	—	—	—	—	—	—	—	—	—	—
10	1 : 10	—	—	—	—	—	—	—	—	—	—
	1 : 20	—	—	—	—	—	—	—	—	—	—
15	1 : 10	++	++	+	±	—	—	—	—	—	—
	1 : 20	++	++	+	±	—	—	—	—	—	—
20	1 : 10	+++	++	++	++	+	+	±	±	—	—
	1 : 20	+++	++	+	+	+	+	±	—	—	—
25	1 : 10	+++	++	++	++	++	+	±	±	±	—
	1 : 20	++	++	+	±	±	—	—	—	—	—
29	1 : 10	+++	++	++	++	++	+	±	±	±	—
	1 : 20	+++	++	++	++	+	+	±	±	±	—
33	1 : 10	++	++	++	+	+	+	±	±	—	—
	1 : 20	++	+	+	+	±	—	—	—	—	—
37	1 : 10	++	++	++	+	+	—	—	—	—	—
	1 : 20	++	++	+	+	±	—	—	—	—	—

++++ to + falling degrees of precipitation
± = trace of precipitation
— = no precipitation

Chester (1936) found that the antigen of tobacco mosaic virus is highly precipitogenic but the healthy tobacco protein complex has a very low order of antigenicity.

Table 3

Precipitation tests with antiserum from rabbit injected with 1 ml of purified sap concentrated 10 times as much as the original dilution. Tests carried out at 5-day intervals with heat clarified sap diluted (1 : 10) and (1 : 20)

Bleeding after (days)	Dilution of virus in saline	Dilution of antiserum and degree of precipitation													
		1 : 2	1 : 4	1 : 8	1 : 16	1 : 32	1 : 64	1 : 128	1 : 256	1 : 512	1 : 1024	1 : 2048	1 : 4096	1 : 8192	1 : 16,384
5	1 : 10	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	1 : 20	—	—	—	—	—	—	—	—	—	—	—	—	—	—
10	1 : 10	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	1 : 20	—	—	—	—	—	—	—	—	—	—	—	—	—	—
15	1 : 10	+	+	+	±	±	±	—	—	—	—	—	—	—	—
	1 : 20	+	+	+	±	±	±	—	—	—	—	—	—	—	—
20	1 : 10	++	++	++	++	++	++	+	+	+	±	—	—	—	—
	1 : 20	++	++	++	++	++	+	+	±	±	—	—	—	—	—
25	1 : 10	+++	++	++	++	++	+	+	+	+	±	—	—	—	—
	1 : 20	++	++	++	+	+	+	±	—	—	—	—	—	—	—
29	1 : 10	+++	+++	++	++	+	+	+	+	+	±	—	—	—	—
	1 : 20	+++	++	++	++	+	+	+	+	+	—	—	—	—	—
33	1 : 10	+++	+++	+++	++	+	+	+	+	+	±	—	—	—	—
	1 : 20	+++	++	++	++	+	+	+	+	+	—	—	—	—	—
37	1 : 10	++	++	++	++	+	+	+	+	±	—	—	—	—	—
	1 : 20	++	+	+	+	±	±	—	—	—	—	—	—	—	—

++++ to + are falling degrees of precipitation.
± = trace of precipitation. — = no precipitation

In our studies it was found that the serum end titre as determined by precipitation tests increases on increasing the dose of purified virus injected into chickens or rabbits. It was also noticed that the serum end titre is usually higher in the case of rabbits than chickens.

This stock material of rabbit and chicken serum served as a quick method for th identification of the tomato streak strain of tobacco mosaic virus.

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Table 4

Precipitation tests with antiserum from chicken injected with 2 ml of purified sap concentrated 10 times as much as the original dilution.

Tests carried out at 5-day intervals with heat clarified sap diluted (1 : 10) and (1 : 20)

Bleeding after (days)	Dilution of virus in saline	Dilution of antiserum and degree of precipitation									
		1 : 2	1 : 4	1 : 8	1 : 16	1 : 32	1 : 64	1 : 128	1 : 256	1 : 512	1 : 1024
5	1 : 10	—	—	—	—	—	—	—	—	—	—
	1 : 20	—	—	—	—	—	—	—	—	—	—
10	1 : 10	+++	++	++	++	+	—	—	—	—	—
	1 : 20	++	++	++	+	+	±	—	—	—	—
15	1 : 10	+++	+++	+++	++	+	±	—	—	—	—
	1 : 20	+++	+++	+++	++	+	±	—	—	—	—
20	1 : 10	+++	+++	+++	++	+	±	—	—	—	—
	1 : 20	+++	+++	+++	++	+	±	—	—	—	—
25	1 : 10	+++	+++	+++	++	++	+	+	—	—	—
	1 : 20	+++	+++	+++	++	++	+	+	—	—	—
29	1 : 10	+++	+++	+++	++	++	+	+	—	—	—
	1 : 20	+++	+++	+++	++	++	+	+	—	—	—
33	1 : 10	+++	+++	++	+	++	+	+	—	—	—
	1 : 20	+++	+++	++	+	++	+	+	—	—	—
37	1 : 10	++	++	+	+	±	—	—	—	—	—
	1 : 20	++	++	+	+	±	—	—	—	—	—

++++ to + falling degrees of precipitation
± = trace of precipitation, — = no precipitation

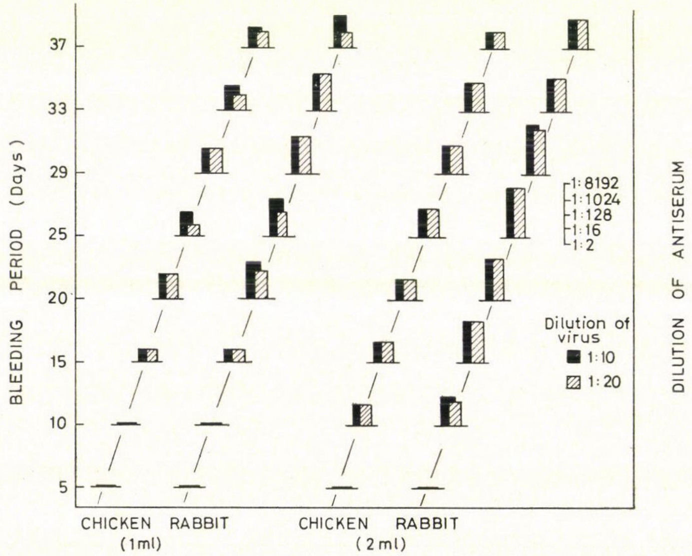

Fig. 2. Serum end titres of chickens and rabbits injected with 1 ml and 2 ml of purified tomato streak strain of tobacco mosaic virus

Table 5

Precipitation tests with antiserum from rabbit injected with 2 ml of purified sap concentrated 10 times as much as the original dilution. Tests carried out at 5-day intervals with heat clarified sap diluted (1 : 10) and (1 : 20)

Bleeding after (days)	Dilution of virus in saline	Dilution of antiserum and degree of precipitation													
		1 : 2	1 : 4	1 : 8	1 : 16	1 : 32	1 : 64	1 : 128	1 : 256	1 : 512	1 : 1024	1 : 2048	1 : 4096	1 : 8192	1 : 16,384
5	1 : 10	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	1 : 20	—	—	—	—	—	—	—	—	—	—	—	—	—	—
10	1 : 10	+++	++	++	+	+	+	+	±	—	—	—	—	—	—
	1 : 20	+++	++	++	+	+	+	±	—	—	—	—	—	—	—
15	1 : 10	++++	++++	++++	++++	++++	+++	+++	++	++	+	—	—	—	—
	1 : 20	++++	++++	++++	++++	++++	+++	+++	++	++	+	—	—	—	—
20	1 : 10	++++	++++	++++	++++	++++	+++	+++	++	++	+	—	—	—	—
	1 : 20	++++	++++	++++	++++	++++	+++	+++	++	++	+	—	—	—	—
25	1 : 10	++++	++++	++++	++++	++++	+++	+++	++	++	+	+	±	+	—
	1 : 20	++++	++++	++++	++++	++++	+++	+++	++	++	+	+	±	+	—
29	1 : 10	++++	++++	++++	++++	++++	++++	+++	++	++	+	+	+	±	—
	1 : 20	++++	++++	++++	++++	++++	+++	+++	++	+±	+	+	±	±	—
33	1 : 10	++++	++++	++++	++++	++++	+++	++++	++	±	—	—	—	—	—
	1 : 20	++++	++++	++++	+++	+++	+++	++	+	—	—	—	—	—	—
37	1 : 10	++	++	++	++	++	+	+	±	—	—	—	—	—	—
	1 : 20	++	++	++	++	++	+	+	±	—	—	—	—	—	—

++++ to + are falling degrees of precipitation
± = trace of precipitation
— = no precipitation

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PHYSIOLOGICAL STUDIES ON SALT TOLERANCE IN PISUM SATIVUM (L.)

II. Mechanism of salt action during germination

It was reported in our previous paper (**Uprety—Sarin** 1974) that in two varieties of *Pisum sativum* soil salinity affects germination, seedling survival and seedling growth adversely while an application of phosfon-D ameliorates these deleterious responses. Salt damage during germination was reported to be brought about by the osmotic inhibition of water absorption or by the accumulation of the constituent ions of saline media and/or by the change in the metabolism (**Bernstein—Hayward** 1958). However, these conclusions on the mechanism of salt action were based on experiments carried out at what may be termed as an 'advanced stage' of germination bacause invariably the seeds were designated as 'germinated' when they showed a measurable length of radicle and plumule. **Mayer—Poljakoff—Mayber** (1963) demonstrated that a large variety of changes start taking place in the seeds immediately after they imbibe water and the subsequent growth of the embryo is the consequence of these changes. Further, **Bonner** (1950) believed that during germination marked changes occurred in the nature and disposition of the nitrogen compounds of seeds which led to the germination process. It was thus apparent that salt damage on germination can be achieved by investigating the changes in nitrogenous constituents immediately after the hydration of the seeds.

Thus in the present series of investigation seeds of *Pisum sativum* (L.) var. *'Vares'* were examined for the changes in their nitrogenous constituents at the initial stages of germination. Further, the study of these changes, on seeds sown in phosfon-D treated salinized soil will provide conclusive evidence for the precise effects of soil salinity during germination by reconfirming the responses on reversing the salt stress. It will also be helpful in providing information on how the effect of phosfon-D was mediated in inducing the salt tolerance during germination.

Seeds of *Pisum sativum* (L.) var *'Vares'* were sown in petridishes according to the technique outlined in the previous paper. Only three series of petridishes were maintained containing a) Unsalinized soil (Electrical Conductivity (E. C.) b) Salinized soil (E. C. of soil saturation extract was 8.0 mmhos/cm. at 25°C) and c) Salinized soil treated with phosfon-D (16 mg. per

300 g of salinized soil), because only an application of phosfon-D was successful in ameliorating the adverse effect of soil salinity on germination and seedling growth.

Adequate samples of seeds were collected at 1, 6, 12, 18, 24 and 30 hours after sowing from each treatment. The samples were analysed for total nitrogen by the colorimetric method of SNELL—SNELL (1949), protein nitrogen according to the method of THIMANN—LALORAYA (1960), peptide and amide nitrogen by BRADY's method (1961), amino nitrogen by the method of LEE—TAKAHANSHI (1966) and proteolytic activity by YEMM—COCKING's method (1955). All the data except those of proteolytic activity were expressed as a percentage of the dry weight of the tissue.

Results on the influence of soil salinity and phosfon-D on various nitrogen forms and protolytic activity are presented in Table 1.

It was observed that the salinization of the soil brought about a marked reduction in the total nitrogen content of seeds and this reduction was apparent 12 hours after sowing but continued for the rest of the experimental period. An application of phosfon-D to the soil resulted in an increase in total nitrogen and this effect was marked only at 24 and 30 hours after sowing.

The salinization of the soil decreased the protein nitrogen of the seeds and this response was again only observed 12 hours after sowing and later. The phosfon-D treatment to salinized soil, however, increased this constituent from 12 hours up to 30 hours after sowing.

Similar to total and protein nitrogen, soil salinity also caused a decrease in the peptide nitrogen level of seeds throughout the experimental period, 12 hours after sowing, while the phosfon-D treatment caused an improvement 18 hours after sowing. Seeds sown in salinized soil showed considerable enhancement in their amide nitrogen level throughout the experimental period. The phosfon-D treatment appreciably reduced the amide nitrogen content of seeds. It was observed that the amide nitrogen content of seeds decreased with the increase in the hours after sowing in all the treatments. Similar to amide nitrogen, soil salinity caused an appreciable increase in the amino nitrogen content of seeds. The increase was marked at later stages i. e. 18, 24 and 30 hours after sowing. Seeds sown in phosfon-D supplied salinized soil showed a marked reduction in their amino-nitrogen level from 6 to 30 hours after sowing.

Soil salinity considerably increased the proteolytic activity of seeds. This enhancement was manifested 6 hours after sowing and continued throughout the experimental period. Further the application of phosfon-D markedly suppressed the salinity induced increase in the proteolytic activity of seeds.

It was also observed that the amino nitrogen content and proteolytic activity of seeds increased with time in all the treatments.

The investigations conducted during the initial stages of germination (1 to 30 hours) to understand the physiological channels of the adverse effect of soil salinity and its amelioration by phosfon-D revealed that soil salinity depressed the total nitrogen, protein, nitrogen and peptide nitrogen contents but increased the amide nitrogen, amino nitrogen and proteolytic activity of seeds even before the initiation of plumule and radicle. The adverse effect of soil salinity on total nitrogen, protein nitrogen and peptide nitrogen of seeds was not distinctly observed when the seeds were sown in soil supplied with phosfon-D. Similarly the application of phosfon-D to salinized soil also lowered the salt induced enhancement in proteolytic activity, accumulation of amino acids and amide nitrogen of seeds even before the emergence of the radicle.

It was, therefore, concluded that salt damage at 'initial stages of germination' was centered around the changes in the protein metabolism of the seeds. The fact that salinity lowered the level of not only the protein nitrogen but also the peptide fraction suggested that there was an inhibition in protein synthesis. Further, a salt induced stimulation of proteolytic activity indicated a faster hydrolysis of proteins. The thinking that salt injury was manifested

Table 1

Interactive effect on phosfon-D and soil salinity on the nitrogen forms and proteolytic activity of pea seeds (var. vares)

Hours after sowing	Total nitrogen, %			Protein nitrogen, %			Peptide nitrogen, %		
	A	B	C	A	B	C	A	B	C
1	5.00	5.03	5.00	4.11	4.10	4.10	0.55	0.55	0.53
6	4.52	4.23	4.30	3.18	3.00	3.00	0.50	0.50	0.45
12	5.25	4.70	4.80	4.00	3.30	4.00	0.65	0.54	0.58
18	5.23	4.69	4.80	4.00	3.50	4.00	0.68	0.52	0.62
24	5.50	4.80	5.00	4.78	3.50	4.52	0.60	0.45	0.55
30	5.50	4.80	5.20	4.90	3.82	4.60	0.61	0.46	0.52

Hours After sowing	Amide nitrogen, %			Amino nitrogen, %			Proteolytic activity mg. leucine/100 g fresh wt/ hr		
	A	B	C	A	B	C	A	B	C
1	0.081	0.080	0.079	0.23	0.23	0.23	130	131	136
6	0.044	0.071	0.064	0.30	0.40	0.35	180	247	200
12	0.038	0.054	0.050	0.32	0.47	0.40	235	370	250
18	0.030	0.059	0.040	0.35	0.49	0.40	260	470	300
24	0.031	0.051	0.041	0.40	0.60	0.48	300	567	332
30	0.032	0.056	0.040	0.45	0.69	0.50	315	600	350

A — Seeds sown in unsalinized soil
B — Seeds sown in salinized soil
C — Seeds sown in salinized soil treated with phosfon-*D*

both in a lowered synthesis and an enhanced hydrolysis of protein was further supported by the observed accumulation of amino nitrogen in seeds sown in salinized soil. These observations indicated that probably the salinity injury at advanced stages of germination was mediated through the scarcity of proteins during the initial stages of germination. The present results also indicated that the ameliorative influence of phosfon-D on germination was presumably channellized through the maintenance of the protein levels. Further, the fact that phosfon-D not only improved the level of proteins and peptides but also decreased the proteolytic activity, suggests that its influence on establishing an adequate level of proteins can be explained both by its supporting protein synthesis and by its protecting their hydrolisis.

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TISSUE CONTENT OF NPK AND Mn IN AMERICAN COTTON AS AFFECTED BY MOISTURE TENSIONS AND FERTILIZATION

The importance of adequate nitrogen fertility and soil moisture in cotton production is well established. However, the response of cotton to nitrogen fertilization is largely dependent on the level of soil moisture (**Scarsbrook** *et al.* 1959).

The solubility of the nutrients present in the soil depends a great deal on the availability of moisture and consequently the absorption of nutrients would be affected by the concentration of the soil solution. The distribution of irrigation water should thus be so designed as to maintain a proper equilibrium of soil solution in the root zone at all the stages of crop growth. Obviously an excess of water does not only amount to a wastage but may leach down the nutrients as well.

Since the moisture in the root zone and nitrogen fertility are related to the growth and yield of every crop a study was undertaken to evaluate the absorption of NPK and Mn in cotton as affected by different moisture stresses and nitrogen supply.

The experiment was conducted at Haryana Agricultural University, Hissar (India) on sandy loam soil. Cotten was grown under combinations of three soil moisture tensions before flowering (t) and three soil moisture tensions after first flowering (t). The three moisture tensions represented by 25, 50 and 75 per cent depletion of available soil moisture indicated the stage of application of water. The field capacity of the soil was 16.0 per cent and the wetting coefficient was taken to be nearly 1/3 of the field capacity. The plots were flood irrigated at the proper time.

Each irrigation plot was divided into 3 subplots and nitrogen was applied at 0, 60 and 120 kg N/ha through C A N (20.5 per cent N). Two replications were used for chemical analysis. A basal dose of 21.8 kg P + 41.5 kg K/ha was drilled before irrigation and actual cotton sowing. The crop was sown on April 20, 1967. Dates of irrigation corresponding to the soil moisture tensions are given in Table 1.

Recently matured leaves 4th from the apex on the main axis taken from 20 randomly selected plants at two stages one after the pre-flowering irrigation schedule and the other after the post-flowering irrigation schedule were analysed for total nitrogen, phosphorus, potassium and manganese. Total nitrogen was estimated by the micro-kjeldahl method and phosphorus by the vandate method. Potash was estimated on a flame photometer in triple acid digest and manganese by the dry ashing-periodate method as outlined by CHAPMAN—PRATT (1961).

1st sampling. The data on the effects of irrigation and nitrogen application on the chemical composition of the cotton leaf have been incorporated in tables.

As the second set of moisture regimes had not been introduced by the time of the first sampling only the N Table is presented for the N, P, K and manganese content of the leaf at this stage.

Nitrogen application significantly increased the N concentration sampled in the leaf from 2.82 per cent under 0 kg N/ha to 3.14 per cent under 120 kg N. The effect was broadly related to the dose of nitrogen applied. The per cent P content decreased with each increment of nitrogen, from 0.51 per cent P under 0 kg N/ha to 0.466 under 120 kg N. However, the concentration of potassium increased from 1.99 to 2.10 per cent with the increasing dose of nitrogen from 0 to 120 kg N/ha. The per cent Mn content in the leaf increased from 137.6 ppm to 142.6 ppm with an increase in nitrogen supply from 0 to 60 kg N/ha; but a further increase in N application i. e. from 60 to 120 kg/ha adversely affected Mn concentration which decreased from 142.6 to 122.4 ppm.

Table 1

Actual dates of irrigation

	Before flowering		After flowering		
t_2	t_1	t_3	t'_1	t'_2	t'_3
12/6	12/6	12/6	5/8	5/8	
11/7			12/8	20/8	12/8
22/7	22/7	29/7	20/8	7/9	
29/7			29/8	27/9	27/9
			7/9		
			17/9		
			22/9	16/10	
			6/10		
			16/10		
4	2	2	9	5	2

The increase in moisture tension from t_1 to t_3 during the pre-flowering period increased the nitrogen content of the leaf from 2.99 to 3.13 per cent. The increase in moisture tension from t_1 to t_2 during the pre-flowering period was reflected in a higher P content of the leaf. But a further increase in moisture tension i. e. from t_2 to t_3 severely reduced the P concentration. Since the plant growth was low at the t_3 moisture level, the P uptake was so affected as to bring down P percentage from 0.527 to 0.454.

The increase in moisture tension during the pre-flowering stage was found to have a depressing effect on the K concentration in leaf tissues.

The increase in moisture tension from t_1 to t_2 decreased the Mn content of the leaf from 133.9 to 129.3 ppm but a further increase in the moisture tension from t_2 to t_3 increased it to 139.5 ppm. There was no indication of any interaction effect of Nxt on the concentration of NPK and Mn in the tissues.

2. Sampling. Nitrogen application significantly increased the nitrogen concentration in the leaf and the increase was commensurated with nitrogen applied. A higher moisture stress maintained during the preflowering period also showed an improvement in the nitrogen content but the effect was significant at the t_3 treatment. Medium and high moisture tensions maintained during the flowering cum fruiting period also raised the N percentage in the leaf, treatment t_2' being the best in this regard. From among the various combinations of t and t' the highest nitrogen percentage (2.76) was noted under t_3t_2'.

The application of 120 kg N/ha raised the per cent P content in the leaf to 0.497 from 0.444 as under 0 Kg N. The effect of moisture tensions maintained during the pre-flowering period still persisted i. e. t_2 increased the P percentage and t_3 decreased it. Various moisture regimes maintained during the later period of crop growth could not cause any significant variations in the P content. However, higher moisture tensions depressed the P concentration in the tissues.

Nitrogen application at 120 Kg N/ha significantly increased the K percentage in the leaf. A medium moisture tension whether maintained during the early or later period significantly depressed the K percentage in the leaf while the low and high moisture tension were nearly similar in their effect.

Increasing doses of nitrogen decreased the Mn content of the leaf under all moisture regimes. The increasing moisture tension during pre-flowering or during the flowering cum fruiting stages also depressed the Mn concentration in the leaf. Among the various combinations of t and t' the highest Mn content was noted in t_1t_2' (74.1 ppm) which was followed by t_3t_1' (73.2 ppm).

Nutrient concentrations in the recently matured leaf on the main axis of the cotton plant (4th from apex) revealed the responsiveness of this crop to nitrogen fertilization as well as to moisture supply. An application of nitrogen significantly increased its absorption consequently affecting the nitrogen congent in the leaf tissues. However, phosphorus concentration decreased with each incremental dose of nitrogen at flowering but increased at maturity.

Dastur (1959) stated that the application of nitrogen increased the absorption of potash provided the latter was sufficiently available in the soil. Obviously with a medium to high availability of potash at the experimental site, an increase was expected in its uptake with nitrogen application. The manganese concentration in tissues at the pre-flowering stage was increased with the application of 60 kg N/ha, but at maturity a decrease in Mn concentration was observed. The results are at variance with those of Mandal (1954) who observed bennefficial effects of N application on the absorption of Mn. There was a fall in the nutrient cum maturity stage, which was apparently due to the utilization of these nutrients in the production of seed cotton (Dastur 1959).

The maintenance of a proper moisture tension in the root zone during the growth, flowering and fruiting period of the cotton crop were found to greatly influence the nutrient absorption. Higher moisture with frequent short interval irrigations before flowering was, it seems, not benefical for the absorption of nitrogen and phosphorus. The highest nitrogen percentage in leaves was observed in the case of 75 per cent moisture depletion while the highest P values were observed in the case of 50 per cent moisture depletion. The higher nitrogen content at the high moisture tension may be due to the high availability of nitrogen in the root zone or else caused by less dry matter production before flowering which is also beneficial for better fruiting and the higher production of seed cotton. In case of post-flowering schedules of irriga-

Table 2

Tissue content of N, P, K and Mn after the first irrigation schedule

	Nitrogen %				Phosphorus %			
Nitrogen level kg/ha	T_1	T_2	T_3	Mean C.D. 0.20	T_1	T_2	T_3	Mean C.D. 0.013
0	2.83	2.86	2.97	2.88	0.475	0.594	0.458	0.510
60	2.93	2.87	3.22	3.01	0.475	0.568	0.453	0.497
120	3.20	3.01	3.20	3.14	0.541	0.419	0.453	0.466
Mean	2.99	2.98	3.13	3.01	0.497	0.527	0.454	0.491
C. D.	0.09				0.022			
C. D. for nitrogen means within a moisture regime								
	0.35				0.031			

	Potash %				Manganese ppm			
	T_1	T_2	T_3	Mean C.D. 0.04	T_1	T_2	T_3	Mean C.D. 0.03
0	2.07	1.90	1.99	1.99	135.7	138.1	139.1	137.6
60	2.08	2.03	2.08	2.07	142.3	136.8	148.7	142.6
120	2.16	2.12	2.03	2.10	123.7	112.9	130.7	122.4
Mean	2.10	2.02	2.03	2.05	133.9	129.3	139.5	134.2
C. D.	0.14				113.6			
C. D. for means within moisture regime								
	0.08				0.376			0.376

Nitrogen level kg/ha	Nxt			Nxt			Nxt'		
	t_1	t_2	t_3	N_0	N_1	N_2	t'_1	t'_2	t'_3
0	2.20	2.37	2.34 t'_1	2.23	2.39	2.61 t_1	2.29	2.58	2.54
60	2.42	2.46	2.59 t'_2	2.50	2.57	2.79 t_2	2.39	2.52	2.62
120	2.79	2.70	2.70 t'_3	2.59	2.52	2.79 t_3	2.55	2.76	2.33
Mean	2.47	2.51	2.54	2.31	2.49	2.73	2.41	2.62	2.50
C. D.	0.06			0.06			0.06		
C. D. for N means with a level of T = 0.10				C. D. for N means with a level of T = 0.10			C. D. for means in the body of table = 0.11		

Table 2

					Phosphorus %				
0	0.475	0.484	0.374	0.428	0.436	0.461	0.462	0.480	0.479
60	0.422	0.491	0.422	0.400	0.427	0.537	0.607	0.419	0.484
120	0.537	0.527	0.436	0.419	0.480	0.484	0.365	0.461	0.400
Mean	0.480	0.502	0.419	0.444	0.449	0.497	0.475	0.453	0.458
C. D.	0.044			0.048			0.044		
C. D. for means within a level of T = 0.088				Within level of t′ = 0.088			In the body of table t = 0.079		

					Potassium %				
0	1.79	1.70	1.88	1.86	1.77	1.83	1.97	1.83	1.77
60	1.82	1.73	1.83	1.68	1.80	1.74	1.68	1.73	1.85
120	1.95	1.83	1.78	1.83	1.81	1.97	1.84	1.67	1.98
Mean	1.85	1.75	1.83	1.79	1.79	1.83	1.83	1.73	1.87
C. D.	0.041						0.041		
C. D. for means within a level of R = 0.058				C. D. within a level of t′ = 0.058			In the body of table t′ = 0.074		

					Manganese ppm				
0	78.7	61.5	59.7 t_1	74.5	65.7	67.3 t_1	68.1	74.1	60.5
60	68.9	54.6	55.8 t_2	61.6	62.0	54.5 t_2	66.2	56.7	53.8
120	55.1	60.5	57.9 t_3	63.9	51.5	51.8 t_3	73.2	47.3	52.9
Mean	67.6	58.9	57.8	66.7	59.7	57.9	69.2	59.3	55.7
C. D.	1.51			2.07			1.51		
C. D. for means within a level of T = 3.76				Within level of T′ = 3.76			In the body of table = 2.60		

N_0 = no, N_1 = 60 kg/ha N, N_2 = 120 kg/ha N.

tion the highest nitrogen content was ob served in t'_2 while the highest P content was noted in t'_1. It very clearly indicates that after flowering cotton needs more frequent irrigation than before flowering. This seems to help better absorption of N and P which would undoubtedly result in enhanced metabolic activities leading to better growth and development. These findings corroborate the observation of **Bruyn** (1964) who also reported the highest cotton yields under high moisture tension, even to the extent of 80—90 per cent moisture depletion. It thus appears that the high nutrient absorption in cotton as reported by **Scarsbrook** *et al.* (1959) was probably not a result of high moisture levels but some other factors.

In case of potash the highest K concentration was noted in high moisture depletion plots. The manganese content was the highest in the t_3 treatment at the 1st sampling stage followed by t_1 but in the post-flowering sampling only 25 per cent moisture depletion (t_1') showed the highest Mn content. This could be due to a high availability of Mn brought about by the high moisture content rendering a low oxygen and high hydrogen tension which is favourable for the reduction of Mn. (SINGH—PATHAK 1967).

It is apparent from this study that under nitrogen fertilization a long gap up to about 75 per cent moisture depletion before flowering and about 50 per cent moisture depletion which under dry conditions may be more than a 20 day interval before flowering and about 15 days after flowering would be an adequate irrigation schedule which will also depend on the climate for the cotton crop. This finding is in close proximity to that of BRUYN (1964) who reported an even higher stress to be equally useful.

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REARING JERSEY CALVES ON DIFFERENT LEVELS AND SOURCES OF ENERGY

There is a persisting shortage in animal production to cover the ever-increasing demand of the growing population in Egypt. The buffaloes and cows are the most important animals for meat and milk production. They provide about 80 per cent of the total meat produced (SALEM 1965).

The importation of Jersey cattle and their grade up with local breed was important in overcoming the animal protein shortage. Jersey cattle proved successful under our local conditions (DARWISH *et al.* 1969). Since the Jersey has a higher ability to grow as compared to the local breed, it is necessary to know the feeding requirements of this imported foreign breed at different ages under our local conditions. Therefore, feeding young Jersey calves during the suckling period was investigated to find out the most economical ration using whole milk along with our common feeding stuffs available in the district.

Nine pure Jersey calves and thirty two crosses with native cattle (23 males and 18 females) were taken from the Animal Production Experimental Station, Faculty of Agriculture, Assuit University. These calves were separated from their dams just after calving and were kept indoors. The experiment was continued until the animals reached 24 weeks of age. At the beginning of the experiment the animals were divided into three groups according to sex and breed. Group I (13 calves) received Treatment "A", Group II (13 calves) received Treatment "B" and Group III (15 calves) received Treatment "C". The whole milk and plant foodstuffs given to different treatments during successive weeks are shown in Table 1. Colostrum was given ab-lib during the first 3 days followed by whole milk from the 4th day up to weaning time. Milk was hand-fed to calves individually twice a day shortly after milking in equal parts at 6 a. m. and 6 p. m.

The plant food stuffs such as clover, clover hay, darawa, wheat straw and concentrate mixture (composed of wheat bran, rice bran, decorticated cottonseed cake, maize and horse bean) were offered from the 3rd week twice daily. The starch equivalents for different plant foodstuffs, roughages and concentrates, were taken after ABDEL-HAFIZ (1969).

Calves were individually weighed weekly early in the morning before feeding.

SNEDECOR's book (1968) was consulted for statistical analysis.

Animals in Treatment "A" were given 523.0 kgs whole Jersey milk, the starch equivalent obtained from such milk was 98.85 kgs S. E. as recommended by JOTTRAND (1957), BOBEK—MOLNÁR (1958) and SIMONJAN (1957), along with 111.44 kgs S. E. plant foodstuffs during the first 24 weeks of age. The total amount of food was 210.29 kgs S. E.

In Treatment "B" the total starch equivalent was reduced to 184.11 by reducing the starch equivalent from milk to 72.67 kgs S. E. (384.5 kgs whole Jersey milk) as recommended by VILLINGER (1956) and LEISNER (1958) and keeping the amount of plant foodstuffs (111.44 kgs S. E.).

The total starch equivalent given to animals in Treatment "C" was 315.44 which was 1.5 times higher than that used in Treatment "A". This was obtained from 784.5 kgs whole Jersey milk and 167.16 kgs S. E. foodstuffs (Table 1).

The average total gain (Table 2) of males was 97.6, 86.8 and 112.2 kgs in Treatment "A", "B", and "C", the corresponding average daily gain was 0.581, 0.517 and 0.668 grs. The birth weight (Table 3) of males in Treatments "A" "B" and "C" was doubled at 52, 70 and 49 days and tripled at 91, 114 and 84 days, respectively. The weaning weight of males became 5.281, 4.602 and 5.857 times their birth weight in Treatment "A", "B" and "C", respectively. The average efficiency of food for males was 2.15, 2.12 and 2.81 in Treatments "A", "B" a "C", respectively.

The relationship between average weight and age was studied using linear regression. The following equations were obtained (Fig. 1).

Treatment "A" $\hat{W} = 15.006 + 4.322\ T$
Treatment "B" $\hat{W} = 13.672 + 3.781\ T$
Treatment "C" $\hat{W} = 12.281 + 4.938\ T$

where W is the predicted (calculated) weight in kgs. at the age T in intervals (one week).

The regression between weight and age is highly significant in all cases and the deviations of the calculated weights were within the allowable limits.

The results of the females indicated that the average total gain (Table 2) was 86.6, 76.8 and 93.7 kgs in Treatments "A", "B" and "C", respectively, the corresponding average daily gains were 0.515, 0.457 and 0.558 kgs. The birth weight (Table 3) of the females in Treatments "A", "B" and "C" was doubled at 63, 70 and 56 days and tripled at 105, 112 and 91 days respectively. The weaning weight of heifer calves in Treatments "A", "B" and "C" became 4.579, 4.589 and 5.074 times their birth weight.

Food efficiencies were 2.43, 2.39 and 3.37 with Treatments "A", "B" and "C", respectively.

Table 1

Feeding chart for weekly allowances of each calf in different treatments during suckling period of the first 24 weeks

	Treatment "A"			
	Whole milk		plant food S.E.	total S. E.
	quantity*	S.E.		
	kg	kg	kg	kg
1–3 days	Colostrum			
4–7 days	12.0	2.268	—	2.268
2	21.0	3.969	—	3.969
3	21.0	3.969	0.84	4.809
4	21.0	3.969	0.84	4.809
5–8	24.5	4.631	2.38	7.011
9–12	28.0	5.292	4.62	9.912
13–16	24.5	4.631	6.02	10.651
17–20	21.0	3.969	6.93	10.899
21–24	14.0	2.646	7.49	10.136
Total	523.0	98.851	111.44	210.291
1–3 days	Treatment "B"			
4–7 days	10.0	1.89	—	1.89
2	17.5	3.308	—	3.308
3	17.5	3.308	0.84	4.148
4	17.5	3.308	0.84	4.148
5–8	17.5	3.308	2.38	5.688
9–12	21.0	3.969	4.62	8.589
13–16	15.75	2.977	6.02	8.997
17–20	14.0	2.646	6.93	9.576
21–24	12.25	2.315	7.49	9.895
Total	384.5	72.674	111.44	184.114
1–3 days	Treatment "C"			
4–7 days	18.0	3.402	—	3.402
2	31.5	5.954	—	5.954
3	31.5	5.954	1.26	7.214
4	31.5	5.954	1.26	7.214
5–8	36.75	6.946	3.57	10.514
9–12	42.00	7.938	6.93	14.868
13–16	36.75	6.946	9.03	15.976
17–20	31.5	5.954	10.39	16.344
21–24	21.0	3.969	11.24	15.209
Total	784.5	148.277	167.16	315.437

Fat percentage 4.5%. It was determined by the classical Gerber method.
* Note: 1 kg whole Jersey milk 4.5% (with mollgard equation (15) = 0.189 kg.S.E.)

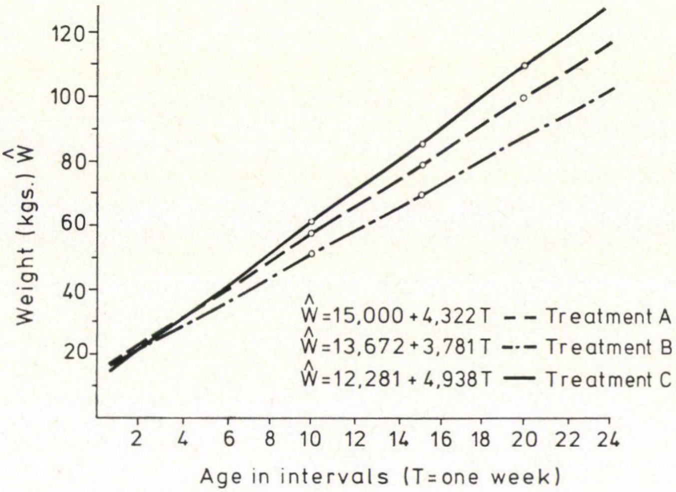

Fig. 1. **Comparison of growth among male Jersey calves reared on different levels and sources of energy during the first 24 weeks of age by linear regression equation**

The relationship between average weight and age was studied using linear regression. The following equations were obtained (Fig. 2).

Treatment "A" $\hat{W} = 14.425+4.009\ T$
Treatment "B" $\hat{W} = 11.656+3.370\ T$
Treatment "C" $\hat{W} = 14.773+4.188\ T$

where $\hat{W}$ is the predicted (calculated) weight in kgs at the age T in intervals (one week).

The regression between weight and age is highly significant in all cases and the deviations of the calculated weights were within allowable limits.

It was found that the analysis of variance between the three treatments, the differences between the treatments were highly significant.

Table 2

Average daily gain and efficiency of food utilization of both sexes in different treatments

Treatment		Birth wt.	Weaning wt.	Total gain	Daily gain	Relative daily gain assuming the highest 100	Food intake	Efficiency of food
		kgs	kgs	kgs	kgs		kgs	
A	Male	22.8	120.4	97.6	0.581	88.03	1.252	2.15
	Female	24.2	110.8	86.6	0.515	77.09		2.43
B	Male	24.1	110.9	86.8	0.517	77.39	1.095	2.12
	Female	21.4	98.2	76.8	0.457	68.41		2.39
C	Male	23.1	135.3	112.2	0.668	100.00	1.878	2.81
	Female	23.0	116.7	93.7	0.558	83.53		3.37

Statistical analysis (Table 4) of the animals' body weight showed that there were highly significant differences between treatments and between periods, while there were significant differences in the body weight of calves between sexes.

The data in Table 5 indicate that the differences in the efficiency of food utilization between the treatments were highly significant while the differences between sexes were significant. The differences between periods were not statistically significant.

From these feeding studies it could be noticed that male calves in Treatmant "A" grew better than animals of both sexes in all treatments except male calves in Treatment "C".

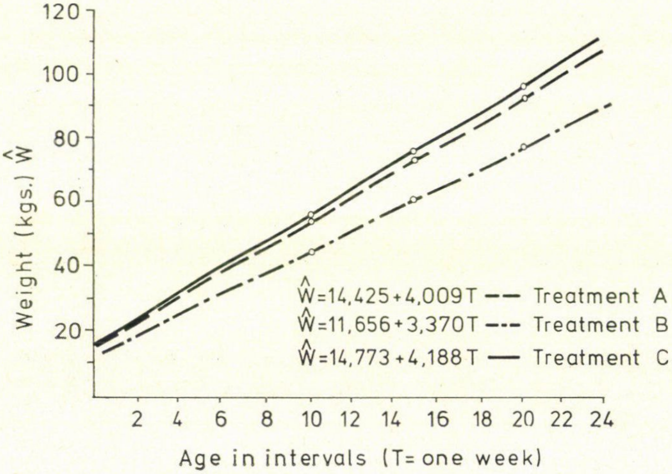

Fig. 2. Comparison of growth among Female Jersey calves reared on different levels and sources of energy during the first 24 weeks of age by linear regression equation

Assuming the total gain obtained during the suckling period by male calves in Treatment "A" is 100, the relative total gain in females of the same treatment is 88.73 per cent, while it is 88.93 per cent and 78.69 per cent for males and females of Treatment "B", respectively. The corresponding values of animals in Treatment "C" are 114.96 and 96.00 per cent.

As the whole Jersey milk in Treatment "B" was 384.5 kgs 73.0 per cent of that in Treatment "A", the total gain was much lower in both sexes than that of male calves in Treatment "A". These results indicated that such allowances were not adequate to let the animals show their full growth capacity.

Although the increased feed intake in Treatment "C" (1 1/2 times that of Treatment "A") caused a slight increase in male calves (114.96 per cent only) compared to male calves in Treatment "A", such an increase in feed was not compensated by a suitable increase total gain. Females (in Treatment "C") failed to obtain the total gain of males of Treatment "A" in spite of increasing the feed intake 1 1/2 times.

From these results it can be concluded that 523 kgs whole Jersey milk and foodstuffs equal to 111.44 kgs S. E., the total starch equivalent being 210.29 kgs, gave normal growth for Jersey calves during the suckling period. Similar results were obtained by Dedeckova-Salova (1969) who fed Czechoslovakian Red Pied calves 211.7 S. E., the total gain obtained during the 24 weeks was 116.7 kgs. While Smilevski *et al.* (1969) fed Dutch Black Pied heifer calves over the 1st 14 weeks of life on 497 kgs whole milk with concentrates and hay ad. lib for 120 days the only total gain obtained was 93.0 kgs. Whitaker *et al.* (1957) found significant correlations between weight gain and concentrate consumption in Jersey calves.

Table 3

Live weight relative to birth weight of male and female calves in the three treatments

Age in weeks	Treatment A				Treatment B				Treatment C			
	male		female		male		female		male		female	
	live wt.	live wt. relative to birth wt	live wt.	live wt. relative to birth wt.	live wt.	live wt. relative to birth wt.	live wt.	live wt. relative to birth wt.	live wt.	live wt. relative to birth wt.	live wt.	live wt. relative to birth wt.
	kg	kg	kg	kg	kg	kg	kg	kg	kg	kg	kg	kg
Birth wt.	22.8	100.0	23.2	100.0	24.1	100.0	21.4	100.0	23.1	100.0	23.0	100.0
1	24.6	107.9	25.3	104.5	25.6	106.2	23.1	107.9	24.9	107.8	23.7	103.0
2	27.5	120.6	27.5	113.6	28.1	116.6	24.7	114.9	26.6	115.2	26.8	116.5
3	29.5	129.4	29.2	120.7	29.8	123.7	26.1	121.9	29.7	128.6	29.4	127.8
4	32.4	142.1	31.5	130.2	31.6	131.1	27.2	127.1	33.2	143.7	31.3	136.1
5	36.0	157.9	35.6	147.1	33.5	139.0	29.0	135.5	37.5	162.3	35.1	152.6
6	39.5	173.2	38.0	157.0	36.4	151.0	31.2	148.8	42.7	184.8	40.6	176.5
7	44.0	192.9	39.8	164.5	38.8	160.9	33.4	156.1	46.2	200.0	43.1	187.4
8	48.2	211.4	43.5	179.8	41.9	173.9	36.3	169.6	50.6	219.0	46.5	202.2
9	53.3	233.8	49.1	202.9	44.0	182.6	39.5	184.6	56.4	244.2	51.9	225.7
10	56.8	249.1	48.7	201.2	48.1	199.6	42.4	198.1	59.8	258.9	54.4	236.5
11	62.9	275.9	56.2	232.2	50.8	210.8	45.6	213.1	63.7	275.8	59.1	256.9
12	64.6	283.3	59.7	246.7	54.1	224.5	48.8	228.0	68.6	296.9	64.3	279.6
13	70.6	309.6	66.4	274.4	57.5	238.6	52.1	243.5	73.7	319.0	67.9	295.2
14	77.6	340.4	71.6	295.9	62.2	258.1	56.3	263.1	78.0	337.7	71.8	312.2
15	76.7	336.4	73.8	304.9	65.9	273.4	60.2	281.3	84.2	364.5	75.3	327.4
16	83.3	365.4	82.2	339.7	70.7	293.4	63.4	296.3	89.5	387.4	80.0	347.8
17	88.0	385.9	83.0	342.9	76.1	315.8	67.7	316.4	94.3	408.2	85.0	369.6
18	91.6	401.8	85.8	354.6	81.2	336.9	71.0	331.8	99.6	431.2	91.2	396.5
19	98.4	431.6	90.7	374.8	85.5	354.8	74.6	348.6	105.8	458.0	94.8	412.2
20	101.3	444.3	95.2	393.4	89.9	373.0	78.4	366.4	110.5	478.4	99.2	431.3
21	106.3	466.2	99.4	410.7	94.1	390.5	83.0	387.9	116.3	503.5	102.8	446.9
22	111.1	487.3	104.0	429.9	98.8	409.9	87.7	409.8	123.2	533.8	107.8	468.7
23	116.6	511.4	107.3	443.4	103.8	430.7	93.1	435.0	129.2	559.3	111.4	484.3
24	120.4	528.1	110.8	457.9	110.9	460.2	98.2	458.9	135.3	585.7	116.7	507.4

It is worthwhile to point out that the same time was required for male calves in Treatment "A" and "C" to double their birth weight. These results confirmed that 210.92 kgs S. E. were adequate for suckling calves especially during the beginning of the suckling period, i. e. when the weaning time was shortened. In that connection LUSSE (1954), NATESOVA (1957) and VILLINGER (1956) found that the same time was necessary to double the birth weight of suckling calves (51—52 days).

Table 4

Analysis of variance in the body weight of calves in the three experiments

Source of variance	Degrees of freedom (D.F.)	Sum of squares S.S.	Variance	F calculated
Total	41	43,674.19		
Between treatments	2	1,044.42	522.21	8.2238**
Between sexes	1	335.21	335.21	5.2789*
Between periods	6	41,345.12	6890.85	108.5170**
Error	32	2,031.92	63.50	

** Highly significant
* Significant

Table 5

Analysis of variance in the efficiency of food utilization of calves in the three treatments

Source of variance	Degrees of freedom (D.F.)	Sum of squares (S.S.)	Variance	F calculated
Total	35	10.4562		
Between treatment	2	4.2245	211.23	11.4305**
Between sexes	1	1.3302	133.02	7.1984*
Between periods	5	2.0826	41.65	2.2540 n.s.
Error	27	4.9892	18.48	

** Highly significant
* Significant
n. s. Not significant

On the other hand calves of both sexes in Treatment "B" required 35 per cent more time than male calves of Treatment "A" to double their birth weight. Moreover, the males and females of Treatment "B" required the same time to double their birth weight. Such results indicated that 184.11 kgs S. E. was not adequate as the animals grew slowly on such allowances which were inadequate for quick growing animals (males) to show their growth capacity.

From these results, it is noted that bull calves in Treatment "A" grew faster than heifer calves relative to birth weight, as males became 5.281 times while females reached 4.579 times (being 87.0 per cent) their birth weight. Moreover, both sexes in Treatment "B" reached the same value of their birth weight as the females in Treatment "A". Such results indicated that the allowances of Treatment "B" were not adequate for males, appeared to be permissible in practice for females and did not impair the growth of female calves, i. e. the slow growing animals (females) would accordingly receive lower requirements than the quick growing ones. Therefore, the requirements in Treatment "B" (13.0 per cent reduction in the total starch value of Treatment "A") would be adequate to allow the quick growing female to show its growth capacity.

Berk—Bedő (1967) reported that rich concentrate and good quality bulk fodder were essential for the successful rearing of Hungarian Red Pied calves on restricted milk ration.

Moreover this would be advantageous, it would reduce the feeding cost of suckling heifers as the whole milk would be reduced by 27 per cent. Kliesch—Horst (1959) concluded that production costs were lower with less whole milk in the ration. Ghoneim *et al.* (1963) claimed that reducing the milk allowances was more economical for rearing Egyptian cow and buffalo calves.

The growth results were reflected in the amount of food utilized, i. e. the efficiency of food utilization was lower with females than that with males in each treatment. Moreover, the higher were the food allowances given to the calves, the lower were the food efficiencies obtained. The worst food efficiency was obtained by females in Treatment "C". The amount of food required for producing 1 kg live weight was 33.6 per cent more than that with male calves in Treatment "A".

Such results indicated that the amount of food given to calves in Treatment "C" was much more than the suitable allowances that produce normal growth. Therefore such allowances were surplus and not economic. On the other hand the amount of food required for producing 1 kg live weight in growing bulls in Treatments "A" and "B" was nearly the same. Such results confirmed the previous data which indicated that the amount of food given in Treatment "A" was adequate while that given in Treatment "B" was not enough as the final total gain was not obtained.

The values of the efficiency of food utilization obtained in Treatment "A" were within the values recorded by Czako (1962) who found that Hungarian spotted calves used a starch equivalent of 2.04, 1.85, 2.42 and 2.22 kgs per kilogram weight gain. Similar results were reported by Adam—Szentmihályi (1961). At the same time Mathieu—Wegat-Litre (1962) found that the food unit per kilogram weight was 3.0 in French Friezian calves fed 200 litres milk during 5 weeks along with concentrates and hay for appetite.

Lusse (1954) reported that the mean amounts utilized per kilogram gain weight during 26 weeks were 1.924, 2.242 and 1.894 kgs starch unit in three groups reared on different amounts of whole milk, skim milk, concentrates and hay.

It was also of interest to find out the relation between weight and age. In the six cases the relation was found to be significantly linear. These results confirmed those already obtained by Salem (1965) for native cattle, proving that from a practical point of view the relation between weight and age was linear. The predicted weights obtained by the equations deviated negligibly from the practical ones.

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EFFECT OF SOIL INSECTICIDES ON PLANTS

III. Effect of certain soil insecticides on the germination of cotton seeds, growth, dry weight, cotton yield and the quality of yield

RAFIC—EL-HENNAWI (1961) indicated that low concentrations (50 ppm) of thimet were apparently more effective on the growth behaviour of cotton plants than higher levels (150 ppm). When the dry weight was taken as a measure of growth, the lower concentration of thimet favoured the accumulation of the dry matter in plants. Treated plants in the different stages of growth possessed higher values of dry matter compared with the control.

LONG *et al.* (1967) reported that small increases in sugarcane growth were associated with the application of chlordane to the soil and it seemed likely that the stimulating effects of chlordane on plant growth might be at least as important as the control of arthropod pests.

HACSKAYLO (1957) showed that final dry weights were higher at a lower concentration of thimet than at a higher concentration as compared with the control. Slight to severe necrotic flecking of the leaves occurred and was correlated with levels of thimet in the substrate. Reducing sugars, sucrase and starch accumulated in young plants treated with thimet, while soluble and protein nitrogen decreased. Thimet tended to cause an increase in the oil content of the embryos at the expense of protein formation.

HOPKINS—TAFT (1965) showed that in furrow treatments at cotton planting, di-syston and phorate adversely affected stands of cotton, but UC—21249 (2-methyl-2 methylthio) propionaldhyde 0-(methyl carbamoyil) oxime did not.

TRANCO *et al.* (1960) indicated that the normal annual soil application of insecticides are, BHC 10.8 kg, lindane 0.4 kg and DDT 3.6 kg. Amounts corresponding to 1.3 and 7 annual applications were applied. With BHC, the three applications reduced yields by 20 per cent, 56 per cent and 100 per cent respectively in a sandy soil. Lindane and DDT had no harmful effect.

Lilly—Fahey (1956) found that BHC was not toxic to cotton plants when absorbed by the roots as a systemic poison.

Roberts *et al.* (1962) showed no apparent effect on the growth of cotton when soil was treated with DDT, BHC, gusathion at the rate of 1, 0.45, 0.375 pound respectively per 1/10 acre.

Gawaad—El-Gayar (1971) indicated that thimet, lindane and temik improved cotton yield when mixed with fertilizers and applied 60 days after germination. Thimet and lindane treatment yielded more than did temik treatment and control. Lindane and heptachlor reduced the growth of roots and stems at concentrations of 10 to 50 ppm.

Thimet, lindane, DDT, temik and heptachlor proved that they had no injurious effect on germination except in the case of thimet, it had a toxic effect at high concentrations (40—50 ppm).

Also the tested insecticides did not affect dry weight except in the case of chlorinated hydrocarbons when used at a high concentration.

Histological studies by Gawaad *et al.* (1968) showed that chlorinated hydrocarbon (lindane) which had a long residual effect showed a bad effect on root tissues at rates higher than 20 ppm.

Thimet was less toxic except in rates higher than 40 ppm, it caused a severe effect on ectodermis and cortex cells.

Effect of soil insecticides on germination, dry weight, and growth of cotton plants. The insecticides tested were applied to the soil at rates of 5, 10, 20, 40, 60 and 100 ppm (= kgs active ingredient/feddan).

Six kgs of air dried sieved soil were treated with tested insecticide concentration. Each dose was dissolved in 100 ml acetone and sprayed on the air dried soil. The treated soil was left to dry for 24 hrs. Each treatment (6 kgs soil+insecticide) was placed in a greasy jar and planted with 20 Menofi cotton seeds. Each treatment was replicated two times. Soil moisture was adjusted to be 75 per cent of its field capacity by weighing the jars every two days and compensating for the water loss. All experiments were conducted under controlled temperature 25 ± 2°C in a greenhouse. 21—30 days later the contents of the greasy jars were emptied. The stems and roots of the cotton seedlings were measured and recorded. The percentage of germination in each treatment was also recorded. Dry weight of cotton seedlings, as a growth measure was assayed by drying plants at 105°C for 24 hrs.

Effect of soil insecticides on cotton yield. Field plots in every experimental locality were treated with different rates of soil insecticides to study the effect of insecticide concentrations on cotton yield. Each treatment was replicated 3 times. Cotton yield from each plot was collected and weighed.

Effect of soil insecticides on yield quality. Fineness, strength, effective length, and mean length of cotton staples of each treatment were studied at the Cotton Testing Administration, Cotton Arbitration and Testing Organization, Alexandria. Also, percentage of short fibers was also tested at the same laboratory.

Detection of insecticide residues present in cotton seeds. Samples of cotton seeds from each treatment were crushed. Samples of 50 gr of crushed seeds were shaken with 150 ml acetone for 30 minutes, filtered and washed with 100 ml acetone. Adults of *Daphnia magna* Straus reared and handled according to the methods described by Frear—Boyd (1967) were used for determining insecticide residues present in the filtrate.

Table 1 shows the type and constitution of the soil used in studying the effect of the tested insecticides on the germination, growth and dry weight of cotton seedlings.

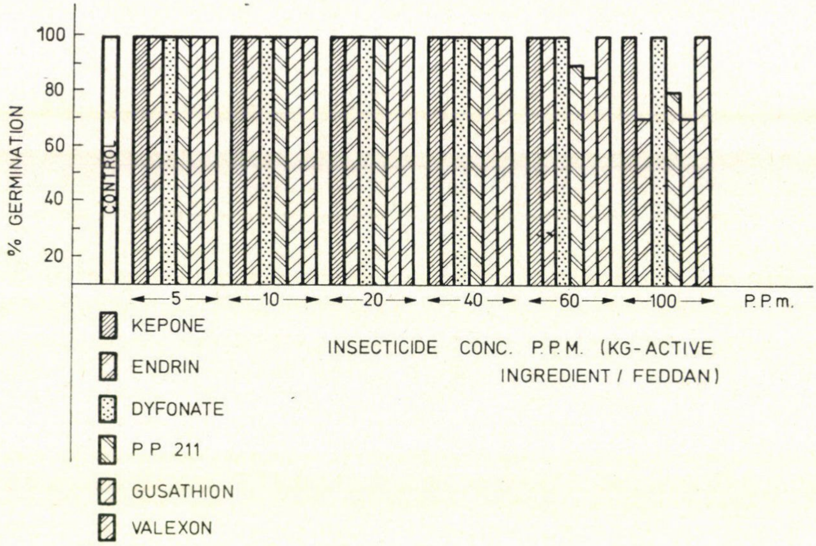

Fig. 1. **Effect of certain soil insecticides on cotton seed germination**

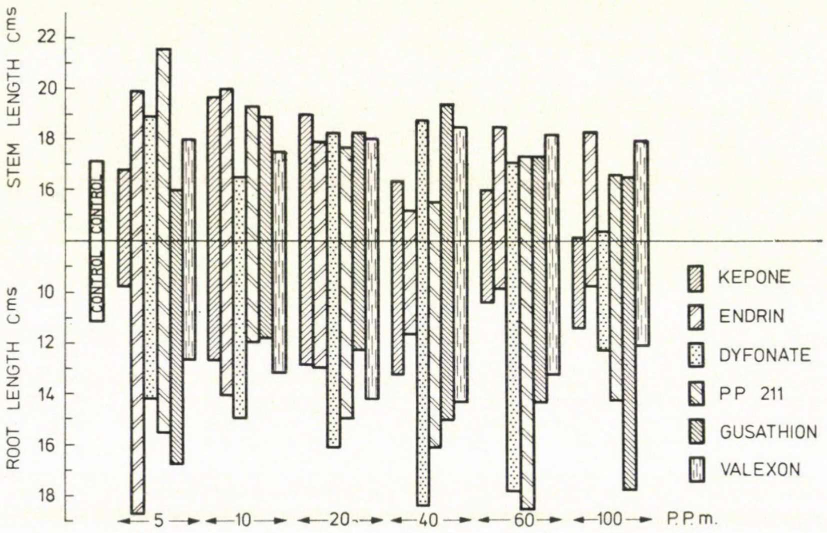

Fig. 2. **Effect of soil insecticides on the growth of the cotton seedling**

Figures 1, 2 show that chlorinated hydrocarbon insecticides (kepone and endrin) had slight effect on growth and germination when used at rates less than 60 ppm. Organophosphorous insecticides enhanced plants' dry weight and growth especially at high concentrations, PP 211 and gusathion suppressed germination percentage when applied at the rate of 60 or more ppm.

Table 2 shows that the tested insecticides might enhance cotton yield when applied to the soil at normal rates, (5 or 10 kgs/feddan) used for controlling the cotton leaf worm. High

Table 1

Mechanical, microbial and chemical analysis of soil used in laboratory

Type of analysis	Contents
1. Mechanical analysis:	
Clay %	39.06%
Silt %	37.15%
Sand %	33.79%
Type of soil	Silty clay loam
2. Microbial analysis:	
Total microflora	4,980.000 per gram
Actinomycetes	730.000 per gram
Fungi	110.000 per gram
3. Chemical analysis:	
pH	8.6
Ca^{++} Mg^{++} mq/L	4.84
Ca^{++} mq/L	3.18
Na mq/L	28.50
K^{+} mq/L	0.39
organic matter	0.77

concentrations of some of the tested insecticides had a bad effect on cotton yield especially at El-Nahda 3 and El-Mansoura, which might be attributed to the soil type of each locality.

Lindane, dyfonate and gusathion were mostly ahead of all the tested insecticides in increasing the cotton yield. Kepone and endrin had slight effect in increasing cotton yield. Valexon 5 kg/feddan when tested at one experimental station only, yielded 15.8 per cent more than the control.

Effects of tested soil insecticides on yield quality are exhibited in Table 3.

Extremely high concentrations of the tested insecticides decreased the pressley index (Strength measure), micronaire reading (fineness measure), effective staple length, mean length of staple, and percentage of short fibers. Lower concentrations had different effects on cotton staple quality. Generally the tested organophosphorous insecticides increased the fineness, effective length of staple and percentage of short fibers, but mostly decreased the strength and mean length of staple.

Results in Table 4 indicate that lindane enhanced lint percentage the most in all treatments. The lint percentages in lindane treated plots in El-Gimmeza, Sakha, El-Mansoura and Etay El-Baroud were 35.9, 39.4, 36.5 and 39.3 per cent respectively. While in untreated plots lint per cent was 34.9, 36.7, 36.1 and 37.7, respectively.

Generally the lint per cent in all treatments at all experimental localities was higher than the controls except at El-Gimmeza treatments treated with 20 kgs/feddan of lindane endrin or kepone.

Table 5 shows that the residues of all the tested insecticides present in cotton seeds were always less than 1 ppm. The tested soil insecticides could be arranged, according to their residual amounts present in cotton seeds in the following descending order: endrin, kepone,

Table 2

Effect of soil insecticides on

Insecticides used	Rate of application kg/feddan	Saft Khaled		Gimmeza		Sakha	
		mean yield per plot	% increase or decrease	mean yield per plot	% increase or decrease	Mean yield per plot	% increase or decrease
Kepone 50%	10	9.646	+ 6.8	8.853	+20.5	15.270	+ 2.2
Endrin 50%	10	10.116	+ 5.7	8.216	+11.2	16.616	+11.2
Dyfonate 5%	5	10.223	+ 7.5	8.256	+12.3	16.500	+10.4
Lindane 5%	10	11.186	+16.9	7.286	— 0.8	16.468	+10.2
PP 211 5%	5	10.750	+12.4	7.201	— 1.5	15.346	+ 2.2
Gusathion 50%	5	9.820	+ 2.6	9.020	+22.8	15.225	+ 1.5
Control	—	9.564	—	7.345	—	14.941	—
L.S.D. .05		N.S.		0.80		N.S.	
Kepone	20	10.776	+17.4	10.406	+31.8	14.330	— 4.1
Endrin	20	10.392	+13.2	10.020	+25.6	14.950	+ 0.0
Lindane	20	11.185	+21.8	9.850	+23.5	15.310	+ 2.4
Control	—	9.177	—	7.970	—	14.945	—
L.S.D. .05		N.S.		0.73		N.S.	
Kepone	40	10.137	+ 7.3	10.560	+22.3	16.180	+13.1
Endrin	40	9.777	+ 3.4	10.016	+16.1	17.962	+25.6
Lindane	40	10.910	+15.4	10.170	+17.8	15.968	+11.7
Control	—	9.447	—	8.633	—	14.295	—
L.S.D. .05		N.S.		N.S.		2.2	
Kepone	60	10.737	+13.9	12.115	+40.7	16.331	+ 9.6
Endrin	60	9.883	+ 4.9	9.817	+14.0	16.687	+11.7
Lindane	60	11.207	+18.9	10.010	+16.2	18.344	+23.1
Control	—	9.422	—	8.605	—	14.900	—
L.S.D. .05		N.S.		1.52		2.4	

dyfonate, PP 211, lindane and gusathion. The results also reveal that no increase in insecticide residues could be detected with the increase in insecticide concentrations from 10 to 60 ppm.

The tested soil insecticides suppressed germination, dry weight and growth when applied to the soil at extremely high concentrations (60 ppm or more). Normal rates of these insecticides 5 to 60 ppm, used for combating cotton soil pests, sometimes favoured growth and enhanced dry weight of plants. Therefore, the tested insecticides could safely be applied as soil treatments for combating cotton soil pests. The effect of the tested chemicals on plant growth was studied using only one type of soil. Further experiments using different soil types must be carried out.

Tranco *et al.* (1960), who used some of the tested chemicals, obtained different results concerning the cotton yield which may be attributed to the difference in the soil type, soil fauna and climate.

quantity of cotton yield

Research Station Faculty of agriculture		High Institute of Cotton		Tiba Farm El-Nahda (2)		El-Mansoura	
Mean yield per plot	% increase or decrease	Mean yield per plot	% increase or decrease	Mean yield per plot	% increase or decrease	Mean yield per plot	% increase or decrease
7.790	− 5.2	4.030	+ 2.2	13.713	+ 3.5	7.583	+ 2.7
8.930	+11.2	4.078	+ 3.7	—	—	7.908	+ 7.1
9.003	+12.1	4.073	+ 3.5	—	—	7.666	+ 3.7
12.290	+53.0	4.331	+ 9.8	15.286	+22.9	9.200	+24.6
8.423	+ 4.8	3.740	− 5.1	14.150	+ 6.9	9.700	+21.4
9.300	+15.8	—	—	17.407	+29.6	10.600	+43.6
8.030	—	3.943	—	13.243	—	7.380	—
1.64		1.20		1.83		1.74	
—	—	—	—	13.063	+ 2.0	7.650	+ 1.3
—	—	—	—	15.950	+24.6	5.500	−26.1
—	—	6.066	+60.5	13.693	+ 7.0	7.256	− 3.1
—	—	—	—	12.797	—	7.482	—
—		—		2.03		1.38	
—	—	—	—	12.903	−11.5	7.080	− 8.5
—	—	—	—	8.993	−41.3	—	—
—	—	3.848	+21.9	13.583	− 5.7	6.645	−14.1
—	—	—	—	14.330	—	7.742	—
—		—		0.77		N.S.	
—	—	—	—	11.333	−16.8	7.470	− 7.1
—	—	—	—	—	—	—	—
—	—	4.550	+44.1	10.393	−23.9	—	—
8.363	—	3.156	—	13.630	—	—	—
—		—		N.S.		N.S.	

Long *et al.* (1967), **Hacskaylo** (1957), **Hopkins—Taft** (1965) commented on the effect of certain soil insecticides on plant growth, dry weight and stand. BHC was recorded to be non-toxic to cotton plants by **Lilly—Fahey** (1956) and DDT, BHC, gusathion were reported to have no apparent effect on the growth of cotton by **Roberts** *et al.* (1962).

The increase in cotton yield in insecticide treatments is due to two factors, first to the probable nutrient effect of certain soil insecticides especially organophosphorous insecticides which enhanced growth (**Gawaad—El-Gayar** 1971, **Gawaad** *et al.* 1970), second to the insecticides' potency against cotton soil-pests which may effect plant growth. Fluctuations in yield experiment results are due to the diversity in soil types and ecological conditions prevailing in each experimental locality.

Soil insecticides are recommended to affect not only the yield quantity but also its quality. Extremely high concentrations of the tested insecticides sometimes had a bad effect

Table 3

Effect of soil insecticides on

Insecitides used	Rate of application kgs/feddan	Pressley index			Micronaire reading		
		Etay	Sakha	Gimmeza	Etay	Sakha	Gimmeza
Kepone	10	9.81	9.07	8.85	3.00	3.65	3.18
Endrin	10	10.54	8.71	9.15	2.60	4.20	2.85
Lindane	10	10.60	9.55	8.97	3.35	4.00	2.95
Dyfonate	5	10.24	10.38	8.95	3.25	4.28	2.98
PP 211	5	9.98	9.85	9.20	3.35	4.10	2.85
Gusathion	5	9.86	10.28	9.32	3.25	4.08	2.95
Kepone	20	10.57	8.48	9.03	3.25	4.30	2.53
Kepone	40	10.04	9.36	9.67	3.05	4.30	2.90
Kepone	60	10.87	8.56	9.35	2.95	4.05	3.25
Endrin	20	10.99	8.66	8.84	3.20	4.05	3.10
Endrin	40	10.73	8.50	8.43	3.20	4.00	3.30
Endrin	60	11.21	5.50	8.87	3.35	3.60	2.75
Lindane	20	10.83	9.88	9.14	2.95	3.90	2.80
Lindane	40	10.62	9.93	9.05	3.28	4.25	3.23
Lindane	60	10.61	9.79	8.51	2.70	3.60	3.25
Control		10.33	10.51	9.44	3.10	3.55	3.15

on yield quantity, while normal rates commonly used for controlling cotton soil pests of some of the tested chemicals improved yield quality.

The tested soil insecticides also proved to enhance lint percentage in all experiments which may be attributed to the loss of seed weight.

Residues of the tested insecticides present in yielding cotton seeds were always less than 1 ppm regardless of the used concentration. This result indicates the possibility of using these cotton seeds in cotton-seed-oil production.

Soil insecticides tested herewith were screened before by the same authors against the cotton leaf worm in bersim and cotton plantations. Their persistence in soil was also discussed, the present study is a continuation of the previous studies dealing with the possibility of the previous studies dealing with the possibility of combating the cotton leaf worm and other cotton pests by using soil insecticides. Further studies of the effect of soil insecticides on soil microorganisms will be discussed later by the same authors to make the study as complete as possible.

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the quality of cotton yield

Effective length			Mean length			Short fiber %		
Etay	Sakha	Gimmeza	Etay	Sakha	Gimmeza	Etay	Sakha	Gimmeza
37.0	37.8	37.0	33.5	33.8	33.5	18.93	19.56	19.41
37.5	37.3	33.8	34.0	33.8	29.5	21.00	18.40	16.34
38.0	39.0	38.3	34.0	35.5	33.0	21.60	21.36	20.15
37.5	38.6	36.8	34.0	34.0	31.5	18.57	25.04	19.66
37.0	39.0	35.0	32.5	35.0	30.8	20.19	23.54	15.84
35.5	39.0	36.8	31.0	35.5	32.3	23.36	21.95	18.76
37.0	41.0	36.8	33.0	37.3	33.0	23.60	19.68	22.70
36.0	38.0	36.0	32.0	34.0	32.3	20.28	18.30	20.51
37.5	37.8	37.8	33.5	33.3	34.3	21.26	20.84	17.11
38.0	38.3	34.8	34.0	33.5	31.0	20.53	21.67	15.21
37.0	36.3	37.3	33.5	31.8	33.0	20.40	18.90	19.09
37.0	37.5	37.5	33.5	32.5	33.0	20.87	17.29	19.37
37.0	37.0	38.5	33.0	37.0	33.5	21.55	21.18	18.62
37.0	36.5	37.5	33.5	36.5	32.5	21.11	20.54	21.26
37.5	36.5	36.0	34.0	36.5	32.3	22.11	20.77	18.09
35.5	39.5	35.5	31.0	36.0	32.8	22.01	20.83	21.47

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Table 4

Effect of soil insecticides on character of staple (lint percentage)

Insecticides used and rate kgs/feddan		Mean lint percentage			
		Gimmeza	Sakha	El-Mansoura	Etay
Kepone	10 kgs	34.9	37.7	37.6	39.3
Endrin	10 kgs	33.9	35.9	34.5	39.2
Lindane	10 kgs	35.6	37.7	36.9	39.1
Dyfonate	5 kgs	35.9	39.3	36.5	39.3
PP 211	5 kgs	35.4	37.3	36.1	40.0
Gusathion	5 kgs	33.7	36.8	36.2	40.9
Control	—	34.9	36.7	36.1	37.7
Kepone	20 kgs	34.7	36.9	35.8	37.3
Endrin	20 kgs	34.6	30.1	36.9	38.1
Lindane	20 kgs	34.7	31.4	36.1	39.3
Control	—	35.8	36.3	35.7	37.9
Kepone	40 kgs	36.2	37.0	36.9	40.4
Endrin	40 kgs	36.6	36.4	—	41.4
Lindane	40 kgs	36.1	36.8	35.2	39.8
Control	—	36.0	36.5	36.0	37.4
Kepone	60 kgs	37.9	37.1	36.6	40.3
Endrin	60 kgs	38.8	35.8	—	39.4
Lindane	60 kgs	37.7	37.8	35.5	39.4
Control	—	35.3	36.3	35.8	37.8
L.S.D.		0.6	0.6	0.7	0.9

Table 5

Insecticide-residues present in cotton seeds

Insecticides X rate kgs/feddan	Residues p.p.m.	Insecticides X rate kgs/feddan	Residues p.p.m.
Kepone 10 kgs	0.378	Lindane 10 kgs	0.105
Kepone 20 kgs	0.648	Lindane 20 kgs	0.154
Kepone 40 kgs	0.676	Lindane 40 kgs	0.124
Kepone 60 kgs	0.720	Lindane 60 kgs	0.134
Endrin 10 kgs	0.700	Dyfonate 5 kgs	0.376
Endrin 20 kgs	0.635	PP 211 5 kgs	0.370
Endrin 40 kgs	0.601	Gusathion 5 kgs	0.100
Endrin 60 kgs	0.820		

EFFECT OF IRRIGATION AND CROPPING ON THE MICROBIOLOGICAL ACTIVITY OF THE SUDAN GEZIRA SOIL

It is generally known that the microbiological activity of the soil is the result of the interaction of several factors such as soil type, climate, previous cropping, moisture conditions, fallowing, cultivation etc. The heavy alkaline cracking clay soil of the Sudan Gezira which lies between the Blue and White Niles (Long. 32°45'—33°55' E, Lat. 13°50'—15°15'N) was elaborately described by GREENE (1928) and FINCK (1961). It is characterized by both its high clay content (40—65%) and low level of organic matter 0.4—0.7%). The clay plan constitutes the main cotton producing area of the country. Cotton (*Gossypium barbadense* L.), Lubia (*Dolichos lablab* L.) and Dura (*Sorghum vulgare* Pers.) used to be grown under irrigation in combination with long fallowing periods. Now wheat and groundnuts are also grown. The "Combined Rotations" Experiment initiated in 1931 contributes one of the series of long-term trials conducted at the Gezira Research Station, Wad Medani, to provide practical information on the evaluation and improvement of crop rotations in the Gezira Scheme. An elaborate description of the experiment was presented by CROWTHER—COCHRAN (1942) and BURHAN—MANSI (1967). It is to be noted that fallowing in the Sudan Gezira generally refers to resting land which is uncultivated.

Long-term trends in rotation responses of cotton in the Sudan Gezira are clearly demonstrated in the work of BURHAN—MANSI (1967) and JACKSON—BURHAN (1968). The "combined rotations" experiment offers a convenient site where the cumulative effect of continuous cropping with one crop like cotton, continuous cropping with more than one crop e. g., cotton — Dura/Lubia and the effect of intervening fallows could be reflected in the soil microbiological status. Earlier (JAGNOW 1964a) reported on cumulative rotational effects on Azotobacter numbers superimposed by irrigation and cropping. The present work was undertaken on some selected rotations to understand the long-term trends in microbiological activity with special emphasis on those related to nitrogen transformations.

The four rotations selected for this study were: — continuous cotton designated (C—C—); cotton alternated by Dura and Lubia (C—D/L); cotton succeeded by Dura and Lubia followed by two fallows (C—D/L—F—F), and cotton followed by three fallows (C—F—F—F). Any of these rotational phases represents one season. It is to be noted that fallows are hand-hoed to keep them free of weeds, Lubia is grazed in situ by folded sheep and that Dura is cut at ground level and removed from the plots. Soil samples were collected 0—20 cms from the surface in early June, by the end of the dry season, from rotational phases which would go under cotton in August of the same year. Samples from the Dura/Lubia half plots were composited and only three replicates of five were sampled. In the absence of an uncultivated and unirrigated control treatment within the experimental layout, the permanent fallow plot (Plot 141, Gezira Research Farm) which is about one mile away from the experimental site was sampled as a reference plot. From the permanent fallow plot three samples were investigated each being a composite of ten subsamples distributed over an area of five acres. Counts of bacteria, spore-forming aerobic bacteria and actinomycetes were determined on soil extract agar (BUNT—ROVIRA 1955), fungi on peptone — dextrose agar (MARTIN 1950) by simple dilution plate methods. Azotobacter was determined on modified Ashby's medium (ABD EL MALIK—ISHAG 1968), nitrifying bacteria on Stephensons medium (STEPHENSON 1949), and denitrifiers on a nitrate mineral salt agar using the most probable number method according to HALVORSON—ZIEGLER (1933). Dehydrogenase activity as an indicator of the microbial status of the soil was determined according to LENHARD (1956) and the comparative occurrence of *Rhizobium* was investigated in a pot experiment using the Legume *Dolichos lablab* as an indicator crop. The nitrification potential of the soil was determined by incubation with and without ammonium sulphate at 75 per cent water-holding capacity and 30°C and

Table 1

Counts of bacteria, spore-formers, actinomycetes and fungi

Rotation	Bacteria ×10^6/gm. soil	Spore-formers ×10^6/mg. soil	Actinomycetes ×10^6/gm. soil	Fungi ×10^3/gm. soil
C—C	6.5 (± 0.24)	0.9 (± 0.05)	2.9 (± 0.27)	26.8 (± 0.61)
C—D/L	10.0 (± 0.35)	1.2 (± 0.03)	3.8 (± 0.31)	24.1 (± 0.87)
C—D/L—F—F	8.9 (± 0.19)	1.6 (± 0.04)	4.2 (± 0.15)	19.9 (± 1.0)
C—F—F—F	7.0 (± 0.15)	2.0 (± 0.06)	3.1 (± 0.19)	14.9 (± 0.8)
Permanent Fallow	4.8 (± 0.21)	1.6 (± 0.02)	2.1 (± 0.23)	15.1 (± 1.1)

carbon dioxide evolution as an indicator of the mineral nitrogen status of the soil was carried out according to CORNFIELD (1961).

Counts of bacteria, aerobic spore-forming bacteria, actinomycetes and fungi are presented in Table 1. It was evident that the irrigated and cropped rotations generally sustained a much higher population of microorganisms than the permament fallow pot. The spore-formers decreased with an increased intensity of irrigation. Furthermore, rotations, with Dura and Lubia, with or without fallows, supported a bacterial population markedly higher than either continuous cotton or cotton followed by three fallows. The proportion of both *Actinomycetes* and aerobic spore-formers in relation to the total bacterial population generally increased with fallowing. Fungi, on the other hand, showed appreciable stimulation with continuous cropping but less so when fallows were introduced in the rotations.

The counts of *Azotobacter*, nitrifying population are presented in Table 2. Counts of *Azotobacter* obtained by the method used, were generally about ten times higher than those reported by JAGNOV (1964b) in which he used thin layers of inoculum dilutions suspended in agar overlying solid nitrogen-free medium. However, the trend in counts pertaining to rotations was similar. The content of *Azotobacter* increased with the irrigated cropping. It was found that the inclusion of Dura — Lubia in the rotation resulted in a many-fold increase in the *Azotobacter* content. The counts of *Azotobacter* tended to decrease with fallowing. Considering the permanent grass fallow as a reference plot, the nitrifiers showed marked stimulation in the rotation with Dura and Lubia and little stimulation with cotton followed by three fallows. Continuous cotton plots, however, were not very different from the permanent fallow. The denitrifiers in the continuous cotton and in the Dura/Lubia plots followed by two fallows showed an increase. Similar to the case of *Azotobacter*, fallowing reduced the numbers of denitrifiers.

Table 2

Most probable numbers of Azobacter, nitrifying and denitrifying bacteria (×10^4/gm soil)

Rotation	Azotobacter	Nitrifying population	Denitrifying population
C—C	5.4	1.2	3.5
C—D/L	9.3	2.3	4.8
C—D/L—F—F	8.1	2.8	3.5
C—F—F—F	5.6	1.8	2.8
Permanent Fallow	3.5	1.3	2.4

Table 3

Nitrifying potential of the soil from various rotations

Rotation	Release of nitrate-N		Co_2 evolution with cellulose in	
	unamended soil ppm No_3—N	soil + 200 ppm NH_4 —N % of continuous cotton	mgs/100	g soil
C—C	18.4	100	4.12	(100)
C—D/L	20.2	138	6.91	(143)
C—D/L—F—F	19.6	125	7.22	(175)
C—F—F—F	16.0	115	5.31	(124)
Permanent Fallow	22.8	92	6.02	(146)
S.E. of mean	(±0.34)		(±0.592)	

The results of the nitrifying potential of the soil as determined by incubation, with and without ammonium sulphate, are presented in Table 3. Release of nitrate nitrogen by incubation without amendment showed that with the exception of rotation C. F. F. F., which gave appreciably less nitrate production in excess over original nitrate, irrigated rotations gave nearly similar values. The permanent fallow soil on the other hand showed a higher level of nitrifying activity. However, when soil samples were incubated with cellulose according to Cornfield (1961), a different picture emerged with continuous cotton giving least nitrification potential followed by rotation C—F—F—F, and highest activity was obtained from rotation C—D/L—F—F followed by C—D/L. The different results obtained using the two techniques for assessing nitrification potential are probably indicative of the differential cellulose activity of micro-organisms superimposed by the decomposition of crop residues. Furthermore, as a result of the differential history of irrigation and cropping both the physical and chemical characters of the soil are likely to be altered (Fadl 1964, Rai 1965). Dubov (1932) and Belchikova (1949) reported that the ratio of the humic to the fulvic acid fraction may vary greatly under different cultivation and irrigation practices. Organic matter containing different ratios of these two main component fractions of humus were found to differ in availability to soil microbes (Kononova 1961).

Enzymatic activity indicated by dehydrogenase is presented in Table 4. Dehydrogenase activity showed marked stimulation in the rotation C—D/L and C—D/L—F—F and was least in rotation C—F—F—F.

Table 4

Dehydrogenase activity of some rotations

Rotation	Dehydrogenase activity (comparative reduction of TTC)
C—C	27 (125)
C—D/L	63 (286)
C—D/L—F—F	54 (245)
C—F—F—F	22 (100)
Permanent Fallow	38 (172)

Table 5

Mean number and dry weight of nodules of Lubia plants

Rotation	Mean number of nodules (3 pots)	Mean dry wt. of nodules (mgs) (3 pots)
C—C	1.2	2.5
C—D/L	36.0	38.5
C—D/L—F—F	25.5	24.2
C—F—F—F	1.8	2.7
Permanent Fallow	3.5	2.5

A study was carried out of the relative abundance of *Rhizobium* sp. nodulating *Dolichos lablab* in the same phases of rotations mentioned above. Lubia was chosen as an indicator crop because it is the leguminous crop in the rotation and at the same time nodulated by a varied group of *Rhizobium* strains (HABISH—KHAIRI 1968). Number of nodules and dry weights per pot are presented in Table 5. The results clearly indicated to abundance of *Rhizobium* in rotations carrying Lubia. The intensity of Lubia cultivation in the rotation had a direct effect on the number of nodules and their dry weight which is indicative of the propagation of *Rhizobium* with the intensity of the legume cultivation. Nodulation in continuous cotton soil and soil from the cotton followed by three fallows was minimal and was slightly less than in the permanent fallow soil.

The above mentioned results pinted clearly to the intensification of the microbiological processes with irrigation varying with the crop rotation and fallows. JAGNOW (1964a) attributed the stimulation of Azotobacter to the nature and amount of crop residues left after harvest. MUKHTAR—MUSA (1967) investigating the effect of roots, stems and leaves of rotational crops on the soil microbial population came to similar conclusions. In the present study great interest was directed to the nitrification—denitrification relationships. Rotations did not usually show the close agreement expected between the numbers of nitrifiers and the ability of the soil to produce nitrate on incubation under set conditions as shown in Tables 2 and 3. This indicated that it was the activity of nitrifiers which was probably different in the various rotations. However, it may be concluded that where Lubia was included in the rotation nitrification was enhanced and that intensified rotations with continuous cotton and C—D/L stimulated the denitrifiers to a greater extent as a result of the supply of heterogenous organic residues as well as excretions from the rhizosphere. These findings are in line with those reported by HARMSEN—VAN SCHREVEN (1955), DELWICHI (1956) and BREMNER—SHAW (1958). Fallowing, though supported fewer nitrifying populations, seemed to sustain high activity and at the same time sustained a comparatively smaller denitrifying population. Besides the change in the nitrifiers some other benefits in the form of the physical improvement of the soil attributed to fallowing (CROWTHER 1943, JONES 1958) should not be ruled out. The permanent fallow soil showed marked nitrate production which is typical of virgin and uncultivated soils when first disturbed, and higher cellulose decomposing activity. Irrigation, on the other hand, increased the capacity of the soil to nitrify added ammonium sulphate as compared to the permanent fallow soil (Table 3). Similar results for Russian soils were reported by KONONOVA (1961).

Enzymatic activity, indicated by dehydrogenase activity was closely related to the general trends of the microbial population. Under optimum conditions, the dehydrogenase activity seemed to be a good indicator of the microbial status of the soil with special bearing on the easily decomposable content soil carbon. Soils which showed low dehydrogenase activity were markedly stimulated on incubation with the addition of a low concentration of glucose but not with the addition of nitrogen (author's unpublished work). This was an indication that the microbial activity of the Gezira soil was restricted more by the short supply of available carbon than by nitrogen. Musa—Mukhtar (1969) reporting on the enzymatic activity of a Gezira soil profile reached similar conclusions.

The effect of rotations on the abundance of *Rhizobium* showed the superiority of rotations including the legume Lubia in relation to rotations with cotton and fallows only and the permanent fallow plot. Inoculation may thus be needed if rotations without a legume such as continuous cotton or C—F—F—F are to support a good crop of Lubia. A preliminary survey of the occurrence of weed flora in fallows outlined the absence of leguminous weeds in the above rotations; the weeds were predominantly graminaceous *(Brachiaria eruciformis)* with some dicotyledonous ephemeral flora showing up during the rainy season. The slight increase in the number of nodules in the permanent fallow plot could be due to the presence of some of the leguminous weeds namely the *Crotalaria* and *Tephrosia* spp. (Bashir 1970).

The performance of these rotations in terms of the yield of the unfertilized cotton (Burhan—Mansi 1967) and of the fertilized coton (Jackson—Burhan 1968) pointed to the superiority of the fallow over Dura and Lubia when immediately preceding cotton. In addition Lubia proved to be superior to Dura in enhancing cotton yields in rotations especially where no fallow preceded the cotton. It was also found that part or all the rotational effect can be reduced by the application of nitrogen. Explanations of both the fallow effect in the rotations having cotton and fallows only and the effect of crops like Dura and Lubia were sought in their impact on soil metabolism. The results of microbiological activity indicated that (1) fallowing generally reduced the intensity of soil microbiological processes namely nitrogen fixation by *Azotobacter*, nitrification and denitrification in rotation with cotton and fallows only (2) fallowing sustains a much higher microbiological activity in rotations with Dura and Lubia. Jagnow (1964a) reported that in one fallow year there is a small change in soil organic matter whereas gains under irrigated phases of cotton, Dura and Lubia were pronounced and records as much as 420 kg N/hectare — 60 cm soil were calculated from changes in organic nitrogen under Lubia, which is a legume, and about half this value under Dura, a non-legume. Explanations could not be found in the *Azotobacter* content alone as numbers were low and the energy status of the soil was not adequate for the heterotrophic activity of non-symbiotic *Azotobacter*. Some form of autotrophic nitrogen fixing activity due to algae was postulated (Jagnow 1964a). Jones (1957) investigating the long-term changes in organic nitrogen and nitrate formation concluded that soil organic nitrogen and nitrate nitrogen in the "combined rotations" did not function in close interdependence which implies either differences in the type of soil organic nitrogen or differences in the physical nature of the soil superimposed by rotation thus affecting the extent of nitrogen transformation. Again, support for the net loss of nitrogen and carbon in the fallow phase could be found in the work of Crowther (1941). In comparing rotations of equal lengths Crowther calculated that over twenty years average yields showed that more nitrogen is utilized in two crops over two years than in one crop and a fallow. This was the case with cotton-Dura and cotton-Lubia, the latter providing excess nitrogen to support another cotton crop. That Lubia resulted in measurable increases in organic nitrogen is not surprising as nodulation was satisfactory and not much of the residues were removed from the plots. However, the deleterious effect of Dura was clearly demonstrated in the three course rotations (Ferguson 1953) whereas in the case of the "combined rotations" with the alternating Dura-Lubia phases the residual effect bestowed by the Lubia seemed to persist and showed itself in good cotton yields in spite of the fact that the Dura residues were low in nitrogen and that they took a long time to decompose (Musa 1965). It can thus be summed up that taking the permanent grass fallow as a reference plot the impact of cropping and irrigation depends on the cropping pattern and that fallowing reduces soil biological activity as a result of both moisture status and scarcity of plant residues. Furthermore, soil microbiological activity namely nitrification and nitrogen fixation were enhanced by irrigation and cropping. Thus the beneficial effect attributed to the fallow does not seem to function through its impact on enhanced soil microbiological activity.

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"MEZŐHEGYESI SÁRGAMAGVÚ" ITALIAN MILLET

Taxonomical place: *Setaria italica* (L.) P. B. convar. *moharia* (Alef.) My.

Origin: Improved by individual selection from a commercial variety grown at Mezőhegyes.

Beginning of breeding: 1974, Mezőhegyes.

Breeders: Pál Bacsa and Vendel Miseta, Kiszombor.

State qualification: provisionally certified improved variety 1956. state certified variety 1963.

General characterization: a resistant variety growing high, developing a thick foliage with broad leaf blades and producing yellow seeds.

Morphological description:

Root system: deep penetrating, strong

Shoot system: 70—120 cm high strong main shoot; the number of laterals may be 1—7.

Stem: light green straw, resistant to lodging; stem diameter: 4,5–5 mm.

Foliage: 18–41 cm long and 8–20 mm broad linear-lanceolate leaf blade. Number of veins 5. The main rib bulges on the lower surface of the leaf. The leaf blade is of dark yellowish green colour, with thin pubescence on the upper surface. On the border of the leaf blade and leaf sheath is the long-bearded ligule. The leaf sheath is covered with a short pubescence, with long beards at the edges. The number of leaves is 9–10.

Inflorescence: cylindrical, slightly bending 15–21 cm long and 1,5–1.9 cm wide panicle. The number of the thickly set panicle branches is 70–135. The main axis and the short branches of the panicle are covered with long hairs. The spikelets are slightly elongated, their length is 10 mm, width 8 mm. The spikelets contain 8–10 flowers, but only a part of them are fertile. The glumes are of light yellowish green colour, their number is 3. The lower glume has 3, the middle 5 and the upper 6 veins. The panicle on the main axis weighs 4 g, the awned grains in it 2.5–3.1 g. (Bányai 1973).

Flowers: closed by the awns; the awns are transparent, of yellowish colour. The anthers are ochre. The stigmas are 2–3 in number, of feathery structure and whitish.

Fruit: grains covered by awns. Thrashing percentage 30–39. The awns are light honey coloured. Thousand-grain-weight 2.9–3.7 g.

Biological characters:

Germination: optimum at a soil temperature of 19–21 °C.

Vegetation period: 110–130 days, with 64–67 days from sowing to flowering (Bányai 1973). Ripening at the end of August, beginning of September.

Water requirement: drought tolerant, with low water requirements (Kapás *et al.* 1965).

Resistance to disease: rather resistant to smut.

Farm technology requirements:

Seeding: optimum in the second half of June.

Soil requirement: nothing special. Reactive to manure applied to the forecrop.

Productivity: grain yield an average of 10.7 q/ha (Csák 1963), green crop 181.4 q/ha on an average.

Region of cultivation: any region of Hungary.

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FORUM

STATISTICAL COMPARISON OF THE ANGLES OF MAIN RIBS IN THE LEAVES AND LEAF REMNANTS OF PLATANUS ACERIFOLIA (AIT.) WILLD. AND PLATANUS ACEROIDES (GOEPP.) HEER (FOSSIL)

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The statistical comparison of leaf parameters in the Tertiary (Sarmatian) platan and the recent *Platanus acerifolia* shows a high similitude between the two plane-trees. This fact and the supplementary investigations suggest that the *Platanus acerifolia* is not a hybrid of either the eastern or the western platan or a progeny of one or the other, but corresponds to the type of the undifferentiated Tertiary primeval platan.

Introduction

The taxon of *Platanus acerifolia* (Ait.) Willd. was first described by W. Aiton (1789) as a variety of *Platanus orientalis* L.: *P. orientalis* L. var. *acerifolia* Ait., and later raised to the rank of species by Willdenow (1805). This order of rank was accepted by W. Town Aiton (1813) in the second edition of the Hortus Kewensis noting that this plane-tree had been introduced in England before 1724 by R. Furber from its native place, the Levant (p. 305).

Spach (1842) summed up the platans described so far under the name *P. vulgaris* Spach, so the above taxon became *P. vulgaris* var. *acerifolia* Spach. He further mentioned that this variety was the most common one in the culture flora.

Loudon (1854), on the other hand, again described this platan as *Platanus orientalis* var. *acerifolia* Ait. (p. 2033), while Jaennicke (1901) again distinguished it as a plant species by the name *P. acerifolia* (Willd.) Jaenn. noting that it may have been only a "forma culta", perhaps a variety of *P. occidentalis* or even a hybrid of *P. occidentalis* × *orientalis*. (p. 121).

Broterus (1804) was the first to describe this plane-tree as a hybrid: *Platanus hybridus* Brot. on the grounds that it seemed to be the hybrid of the eastern and western platan. After its leaves, however, he thought that it may have been a variety of *P. orientalis* (p. 487).

According to De Candolle (1864) this plane-tree — regarding its leaves — is close to the western, and even more so to the eastern platan from which sometimes it can hardly be distinguished (p. 159).

The few examples given above clearly show the uncertainty and contradictions having existed — and still existing — in science or even in practice (horticulture, public opinion) concerning the identification and taxonomy of this and the other plane-trees.

The primeval platan *Platanus aceroides* was first described by Goeppert (1855) from the Tertiary flora of Schossnitz with four and six resp. other primeval platan species (p. 20—22.); these species were summed up by Heer (1856) in such a way that the name *P. aceroides* (Goepp.) Heer even got a different meaning from that in Goeppert (p. 71).

Goeppert pointed out that the fossile platan species described by him were so similar to those existing in his days that one would have been inclined to think them identical (p. 20).

Heer, too, said that one variety of the contracted fossile platan species was closely related with *P. acerifolia*, while the other varieties corresponded — in his opinion — to *P. occidentalis* (p. 74).

According to SAPORTA (1881) the equivalent of *P. aceroides* Goepp. is a native of East-Asia, and the platan indicated a mild, wet climate at the beginning of the Pliocene (p. 317).

Since in Hungary, due to the favourable conditions of fossilization, a great many leaf remnants of primeval plane-trees have been found in the Sarmatian vegetation of water-side valleys Andreánszky (ANDREÁNSZKY 1959) is of the opinion that only one platan species lived here at that time, generally called *P. aceroides* Goepp., further, that this platan was the primeval form of the eastern and western platan species (pp. 75—76). This opinion of his is confirmed in a subsequent work (ANDREÁNSZKY 1965).

The origin of the platans was dealt with by JANKÓ (1889). He studied the first leaves of young developing spring, summer and autumn shoots of a single tree of — supposedly — *P. orientalis* L., then compared them with the drawings of leaf remnants of primeval platans described so far from a comparative morphological point of view. He thought to have found leaf forms typical of both the existing and primeval platans in progressing phases of the vegetation period.

Jankó described the plane-tree species *P. acerifolia* Willd. as *P. orientalis* var. *acerifolia* (Ait.), and characterized the primeval platan *P. aceroides* Goepp. as a less developed form of *P. occidentalis* L. (p. 435). Otherwise, in Jankó's opinion *P. hybridus* Brot. is a synonym of *P. occidentalis* L. (p. 456).

In his investigations Jankó finally arrived at a point where he declared that the traditional ("classic") concept of species could not be applied to the platans, and that he only used the categories of species-variety-form for the purpose of taxonomical description and distinction (p. 447).

It must be noted here that the above mentioned primeval platan was subsequently found not only in the Hungarian Pliocene (see the phytopaleontological collection in the Hungarian Natural History Museum) but also in the Bulgarian Pleistocene (Flora Bulgariae, 1970. IV. p. 617).

It was by the afore outlined situation and above all by the numerous Sarmatian leaf imprints found in Hungary as well as the results of investigations into the Sarmatian flora and vegetation (ANDREÁNSZKY 1959) that the author was encouraged to make biometric comparisons between the *P. acerifolia* (Ait.) Willd. and *P. aceroides* (Goepp.) Heer taxons.

Material and Method

The material chosen for the comparison of the two taxons consisted of leaves from the cca. 200 years old platans of the Budapest Town Park and from the much younger plane-tree alley at Gizella-telep, Visegrád, on the one hand (Plate I,) and of the mostly fractional lower and upper Sarmatian platan leaf imprints kept in the phytopaleontological collection of the Hungarian Natural History Museum.

The Sarmatian leaf specimens (26 of the lower, 19 of the upper Sarmatian) found at North-Hungarian sites distant from one another were chosen for reasons to be mentioned later. (These leaf imprints sometimes show the upper, sometimes the lower leaf surface.)

As the field-work was not primarily aimed at obtaining perfectly intact platan leaves from the layers — since the family of platans belonging to a single genus and species and a relatively unchanged water-side phytocenosis cannot contribute much information to the phytopaleontological researches — the leaf imprints are rather fragmental. Nevertheless, the branching of the main ribs — which is of decisive importance in identifying the finds — has remained relatively intact. At the same time the deviation of the lateral rib — on the recent specimens — proved to be a characteristic leaf parameter in accordance with the proportions

Plate I

Fig. 1. Two *P. acerifolia* (ait.) Willd. trees from the Budapest Town Park; *Fig. 2.* Alley of *P. acerifolia* (Ait.) Willd. at Visegrád, Gizella-telep; *Fig. 3.* Peeling trunk and fruit balls of a plane-tree at Visegrád; *Fig. 4.* Fruit spikes of a plane-tree at Visegrád

of the total length and width of the leaf blade. (When measuring the angle of deviation, on the lower leaf surface always the right-side, while on the upper leaf surface the left-side lateral main rib was taken in consideration.)

Since the leaf imprints were obtained from ten different sites they fulfilled the requirement of random sampling.

The afore mentioned platan leaves (100–140 in number) of the Budapest Town Park and Visegrád were collected — similarly at random — from a mass of fallen leaves in the autumn of 1973.

The angles were measured partly by a plastic triangle openable at one side, partly by a common semicircular angle gauge since the former can only be used up to 45 degrees (quarter-, half- and three-quarter degrees were determined by estimation).

For the respective recent platan leaves the number of the leaf lobes and of the teeth in the middle lobe, as well as the shape of the leaf base and of the sinuses at the leaf margin — that rendered it possible to establish their percentage occurrence — were recorded.

Plates II–IV illustrate simple development forms characteristic of the leaf base of *P. acerifolia* (Ait.) Willd. though they most frequently occur specimens combinations. As a mere control — in the absence of a sufficient number of original in various — angles enclosed by the main ribs in the drawings of *P. orientalis* L. and *P. occidentalis* L.were also measured in the monograph of Jaennicke (1901).

For the statistical calculations relevant tables of the work by Cavalli–Sforza (1965) and Fischer–Yates (1957) were used.

Results

In the platan monograph of Jaennicke (1901) measurements of the length of the leaf, the upper and lower lobe of the leaf as well as of the petiole, further, of the length of one upper and one lower lobe per each with the ratio of the two latter expressed in fractional form are given for *Platanus acerifolia* and the other platan species alike (p. 213). Only the total length of the leaf was not compared to its largest attained width, nor was the fractional value of their ratio calculated.

That is why he could not notice that the fractional value of the ratio between the total length and largest width of the platan leaf increased in proportion with the decreasing values of angles enclosed by the respective main ribs. (This observation has been proved right by numerous random tests.)

Some angles and the fractional values of the corresponding ratios in platan leaves from Visegrád are given below:

30°	$\frac{12.8}{10}$	1.280
33.5°	$\frac{11.3}{10.3}$	1.122
37.5°	$\frac{14}{15.5}$	0.903
44°	$\frac{15.3}{17.2}$	0.889
51°	$\frac{15.3}{21}$	0.728
53.25°	$\frac{18.5}{27}$	0.685

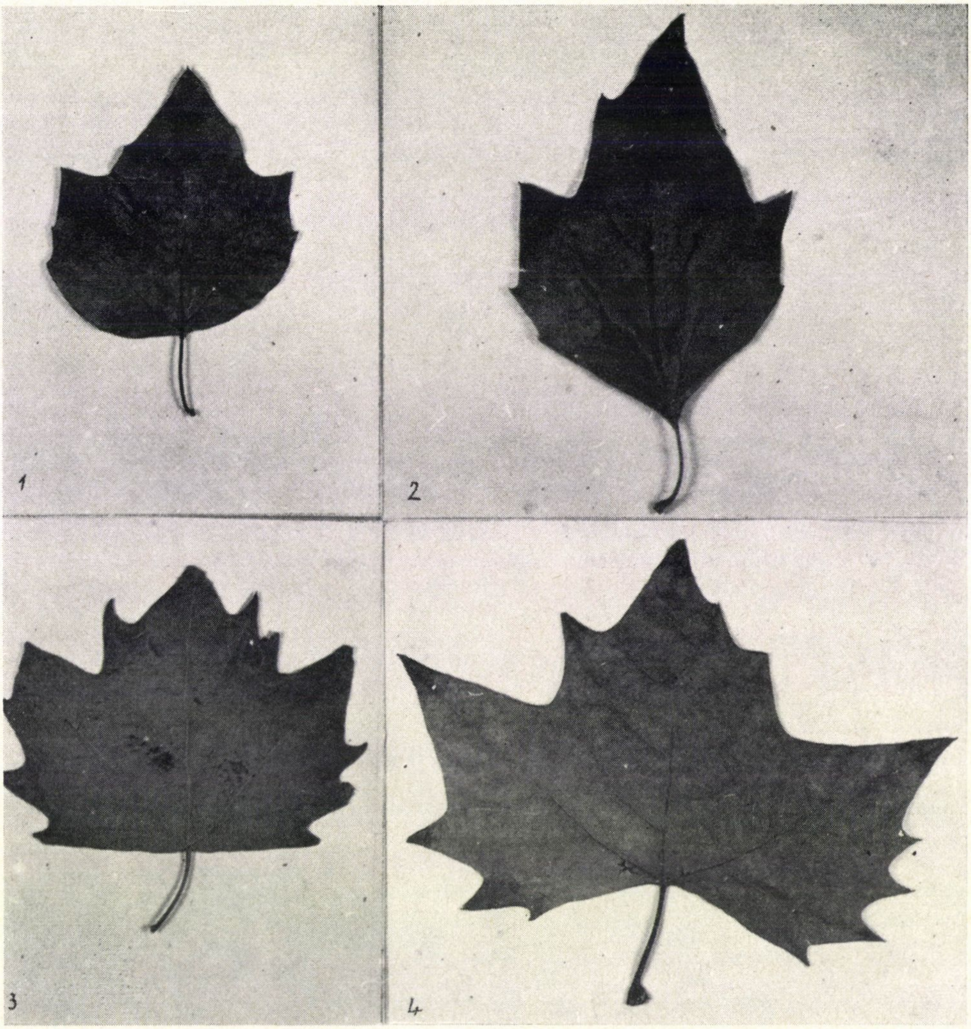


Plate II

Fig. 1. Round-shaped leaf base; *Fig. 2.* Wedge-shaped leaf base; *Fig. 3.* Cut-shaped leaf base; *Fig. 4.* Down-bowing or subcordata shaped leaf base

On the basis of these measuring data and calculations the informative i.e. parameter value of the rib angles has become obvious. It is particularly important because the angles enclosed by the main ribs can be measured on the mostly fragmental leaf imprints of primeval platans too. (With an openable triangular angle gauge even if the point of branching is missing.)

In the absence of decisive diagnostical morphological characters the platan studies attach importance to the number of lobes and shape of the leaf base rather than to the depth of the sinuses at the leaf edge, or the number of teeth, etc.

The percentage distribution of the number of lobes and shape of the leaf base in the 140 leaves of *P. acerifolia* (Ait.) Willd. from the Budapest Town Park, when compared with that in the identical "species" Visegrád platans shows a great variability (Tables 1—2).

Table 1

Percentage distribution of the number of lobes and shape of leaf bases in the plane-trees of the Budapest Town Park

Number of lobes	0	3	5	7
Distribution	0%	35%	65%	0%

Leaf base	Round	Wedge-shaped	Cut	Subcordata	Combinations
Distribution	1.5%	1.5%	15.3%	45.7%	36%

The aspects for the morphological classification of leaf bases are seen in Fig. 1. It must be noted that the platan leaf sometimes shows a state of overdevelopment, when before the petiole, or even on the petiole itself a small lobe either connected with or separated from the leaf blade is formed, and transitions may exist between the outlined forms of leaf base too.

The more complex form of the leaf base may mean at the same time the increased then tree too. On the other hand, often there are larger leaves with thinner and softer tissues and

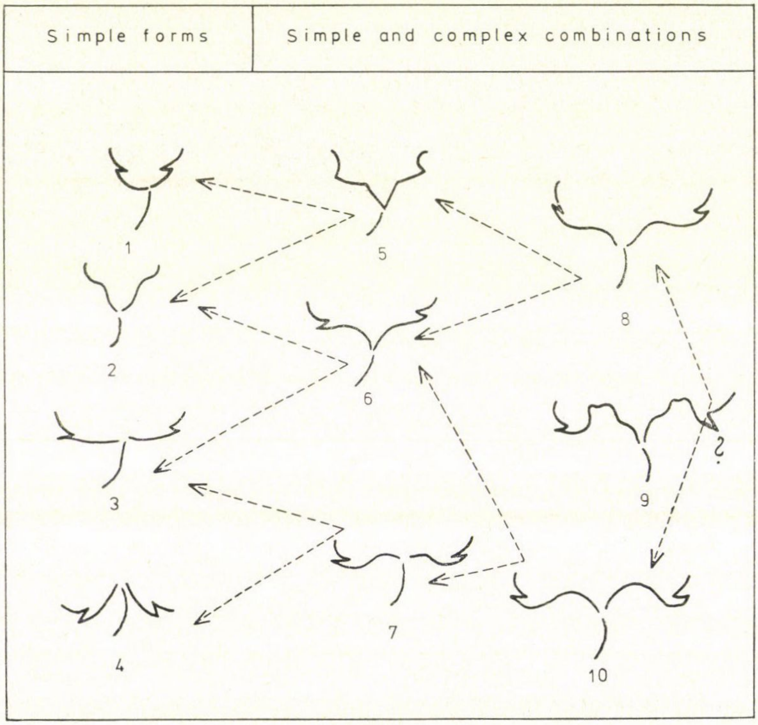


Fig. 1. Outlines of leaf base forms. (1 = round; 2 = wedge-shaped; 3 = cut; 4 = subcordata; 5 = round-wedge; 6 = wedge-cut; 7 = cut-subcordata; 8 = round-wedge-cut; 9 = round-wedge-cut-subcordata; 10 = wedge-cut-subcordata)

Table 2

Percentage distribution of the number of lobes and shapes of leaf bases in the platan trees of Visegrád

Number of lobes	0	3	5	7
Distribution	0%	45%	55%	0%

Leaf base	Round	Wedge-shaped	Cut	Sub-cordata	Combinations
Distribution	5%	30%	21%	24%	20%

less developed shape, or more developed but smaller leaves with thicker, harder tissues. The latter phenomena are certainly caused by the influence of physiological or ecological factors.

The use of leaf imprints from the Sarmatian primeval platan in statistical comparisons was not only due to their frequent occurrence in Hungary or the recognition of the parameter nature of the rib angles.

The work of ANDREÁNSZKY (1959) on primeval flora and vegetation uncovered almost completely the Sarmatian woody flora of this area. He could also establish that no such extent of change had occurred either before or after in the pattern of vegetation while the primeval platan as a gallery forest tree remained fairly unchanged. On the ground of his investigations he assumed that the Sarmatian conditions east of Hungary had set in later, and the Hungarian Sarmatian flora corresponded to the Pliocene flora of the Balkan Peninsula (p. 312—320).

Thus the survival of the primeval platan in the Mediterranean region is easily imagined and made — otherwise — probable by the afore mentioned Bulgarian finds of Pleistocene primeval platans. All this increases the informative value of the Hungarian imprints of primeval platan leaves.

The statistical comparisons were made in the following way:

The frequencies of rib angles measured in *P. acerifolia* (Ait.) Willd leaves obtained from two different places (Budapest, Town Park — Visegrád, Gizella-telep) were grouped in classes with identical intervals. The class intervals were replaced by class means, while the actual mean values by arbitrary mean values ("estimated average") (Table 3).

The mean value and standard deviation of *P. aceroides* (Goepp.) Heer leaf imprints were determined by a digital computer (45 in number).

The variation range of rib angles in 45 leaves of the Sarmatian *P. aceroides* (Goepp.) Heer was 24.75—48°; the mean value: $\bar{x} = 37°$ and the standard deviation: $s = 6$.

The comparison of these statistical data of *P. aceroides* with the corresponding values of *P. acerifolia* from the Town Park seems to confirm a statement by JANKÓ (1889) namely, that in the platan leaves the angle enclosed by the central and lateral main ribs has gradually increased with the advancement of the geological epochs—thus since the Sarmatian too (p. 432).

However, the variation range of the rib angles measured in 104 leaves of *P. acerifolia* from Visegrád was 23.5—52° with a mean value of $\bar{x} = 37.52°$ and standard deviation of $s = 7$.

This surprising result rendered further investigations necessary. The average height of *P. acerifolia* trees examined in the Town Park was 29 m, the average girth at chest height 4.3 m, while the plane-trees of Visegrád had an average height of 40 m and average girth of 2.2 m only.

Table 3

Mean value and standard deviation of P. acerifolia *(Ait.) Willd. leaves from the Budapest Town Park (range of variation: 35—63°)*

	Class interval	Class average	f	X	fX	fX^2
$f =$ frequency	35—38°	36.5	2	−4	− 8	32
$X =$ variant corresponding to the class average	38—41°	39.5	8	−3	−24	72
$A =$ work mean	41—44°	42.5	9	−2	−18	36
$M =$ estimated average	44—47°	45.5	7	−1	− 7	7
$R = 3$	47—50°	48.5	33	0	0	0
$m =$ actual average ($m = A + RM$)	50—53°	51.5	24	+1	+24	24
$C =$ correction member						
$M = SfX/Sf = 80/124 = 0.645$	53—56°	53.5	19	+2	+38	76
$m = A + RM = 50.43$	56—59°	56.5	16	+3	+48	144
$C = (SfX)^2/Sf = 51.61$	59—62°	59.5	3	+4	+12	48
$SX^2 = (SfX^2 - C)/(Sf - 1) = 3.759$	62—65°	62.5	3	+5	+15	75
$SX = \sqrt{SX^2} = 1.94$					SfX	SfX^2
$Sx = R \cdot SX = 5.82$			$Sf = 124$		80	514

Plate III/a

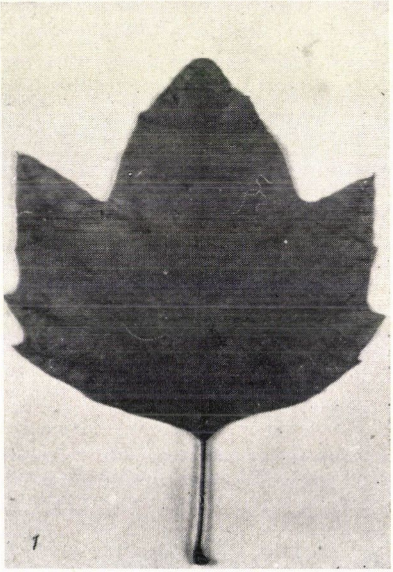


Fig. 1. Round-wedge shaped leaf base

Plate III/b

Fig. 2. Wedge-cut shaped leaf base

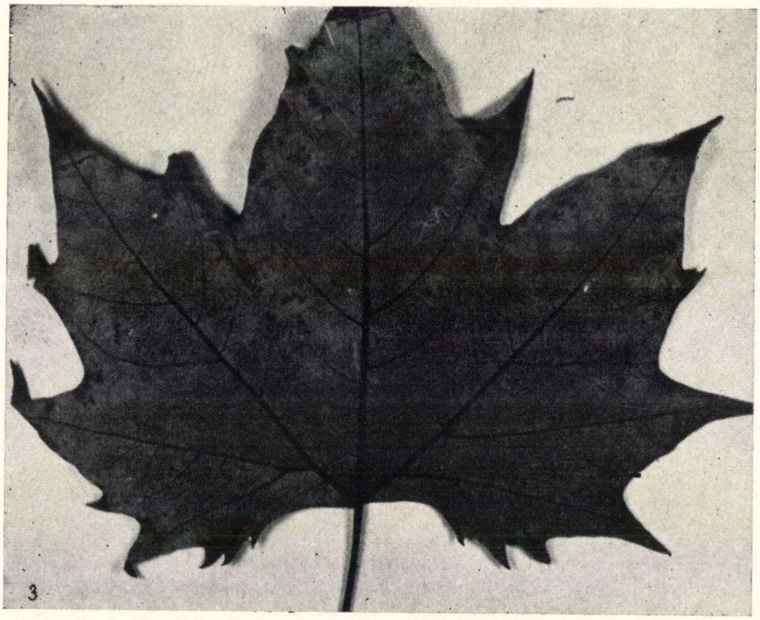


Fig. 3. Cutsubcordata shaped leaf base

The larger height of the obviously younger Visegrád plane-trees was certainly due partly to their spacing of 4.5 × 6 m, partly to the deep valley of north-west — south-east direction in the Danube-bend and the favourable water supply of the gravel terrace. The Visegrád platans are further characterized by the occurrence of fruits set in threes and fours on the same peduncle.

The recent *P. acerifolia* and the fossile *P. aceroides* — as regards their leaves — do not seem to be essentially different even under the given conditions (for the time being primeval platan leaf imprints and sufficiently intact finds are relatively few in number).

It was thus justified — considering the probability of a normal distribution (the straight line of the cumulative frequency distribution on the probability paper, and the low value changes of angles) — to compare the mean values of rib angles on the Visegrád plane-tree- and Sarmatian primeval platan leaves, and their standard deviation with the Student *t*-test and the F-test (Fischer), respectively, at a 5% level as well. The differences were not significant in these cases either, so the averages from the two variation ranges and the difference in standard deviation were only accidental: the samples belonged to the same basic lot.

As a control the angles enclosed by the main ribs were measured in 15 leaves of *P. occidentalis* too, and their mean value ($\bar{x} = 48-69$) and standard deviation ($s = 7$) compared with the corresponding values of *P. aceroides* by the *t*- and F-test. According to the *t*-test the mean values of the rib angles were significantly different, while the standard deviations were not. Naturally — in the absence of original samples — only Jaennicke's supposedly precise drawings reduced to a quarter could be taken for a basis. So these data are only of informative character. It must be further mentioned here that the American or western plane-tree may even reach a height of 50 m (North American Fl. 1908, p. 229).

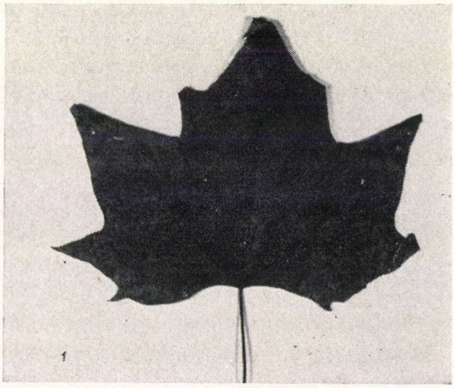
Conclusions

The angles enclosed by the main ribs characteristic of the leaves of the high Visegrád *P. acerifolia* (Ait.) Willd. agree with the corresponding data of *P. aceroides* (Goepp.) Heer. The mean value and standard deviation of rib angles in the leaves of the lower average height *P. acerifolia* (Ait.) Willd. examined in the Budapest Town Park do not significantly differ from those of the Sarmatian primeval platans either. The recent *P. acerifolia* (Ait.) Willd. is therefore supposed to correspond to the type of the undifferentiated primeval platan. This supposition may prove to be a useful work hypothesis in further investigations on speciation, as well as from cytological, cytogenetic and biochemical points of view. It probably will open up new vistas in the research of certain "*pleomorph*" hybrids.

The recent *P. acerifolia* (Ait.) Willd. trees are probably the ones most similar to the Sarmatian primeval platan. According to ANDREÁNSZKY (1959) the primeval platan described by him was the highest of all deciduous trees at that time on the area of his investigations, only exceeded by certain pine-trees, first of all the *Sequoia* (p. 76). For the height probably the warm, wet climate, the more fertile soil, the water-side occurrence and the deep valleys were responsible. (It may be mentioned as an analogous case that in the corrosion heart preparations of animal species running well and persistently, which accurately show the original branching of veins, the angle of deviation is smaller and sharper which is related with the quicker blood circulation, that is blood and oxygen supply, respectively. Probably — ceteris paribus —, the more intensive evapotranspiration in certain tree species is in interaction with the smaller angle of branching.)

The taxonomic classification of the recent platan species will have to be carried out on the grounds of the principles and procedure of numerical taxonomy.

The main objection to the hypothesis outlined in the paper is: how the Sarmatian primeval platan was able to survive till now when only a few unreliable data have been available



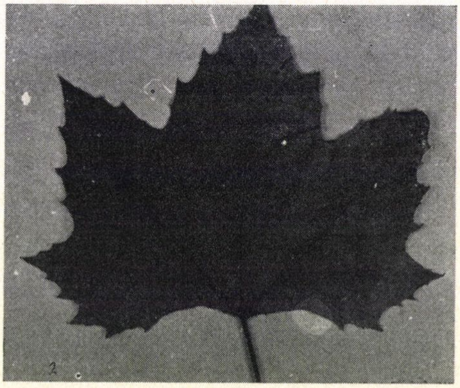


Plate IV

Fig. 1. Round-wedge-cut shaped leaf base; *Fig. 2.* Round-wedge-cut-subcordata shaped leaf base; *Fig. 3.* Wedge-cut-subcordata shaped leaf base

so far on the natural occurrence of the recent *P. acerifolia* (Ait.) Willd. From a later edition (1741) of Plinius' work it is known that the pathway of the platan culture was: Lycia—Cyprus—Crete—Athens—Italy. Plinius made distinctions between the plane-trees of Asia Minor, Lycia and Lydia, and among others spoke about the Moors having had platans in their forests (*"Inter silvas suas Morini platanonas habebant"*) (p. 655). The *P. acerifolia* trees supposedly offered a better shade than the *P. orientalis* that had leaves with deeper indentures, and in the hot southern regions this was an important point of view. It is a well-known fact too, that the plane-tree is estimated to be about 2000 years old. The popular plane-tree was probably propagated in the Roman Empire too. It is only from the 16th century — after the dimness of the Middle Ages — that written documents have remained. Jankó (1889) mentioned after Van Hulthem that Clusius had been given a platan seedling in Vienna in 1576 (p. 412). We do not, however, know what kind of plane-tree it was. It was not even sure in this very century whether it was the western or the eastern plane-tree that was a native of Europe (Mayr 1906 p. 491.), until Jaennicke pointed out after thorough investigations that *P. acerifolia* is the most frequent cultivated European plane-tree.

Our hypothesis seems to eliminate all uncertainties and contradictions in identifying the different platan species and varieties. All we have to do is to put the *P. acerifolia* to its right place, i.e. beside *P. aceroides*. *P. acerifolia* is neither a hybrid of the western and eastern plane-tree, nor a variety of one or the other of them, but a representative of the Central and South-European Sarmatian platan species having survived under specific conditions.

The primeval platan of the loose gallery phytocenoses was apparently forced to change to a considerable degree by isolation only, while that removed by man from its natural environment and phytocenosis has remained, as *P. acerifolia* (Ait.) Willd., more or less unchanged.

Finally, we have to mention the result obtained by Ferguson (1971) in his phytopaleontological investigations on platan species. He did not think a hybridization between P. orientalis and P. occidentalis likely (Johnson 1933 in Ferguson). On the basis of Middle-Miocene platan remnants from West-Germany he thought the primeval platan found there to have been an ancestor of the platan species of today in which the characters of the latter had not yet been fully developed (p. 157). He even proposed a new name for this primeval platan: *Platanus platanifolia* (Ett.) Knoblach, of which *P. aceroides* (Goepp.) is a synonym. In our opinion it is not the primeval platan but the *P. acerifolia* (Ait.) Willd. whose name probably will have to be changed, as it is a possible variety of *P. aceroides* (Goepp.) Heer.

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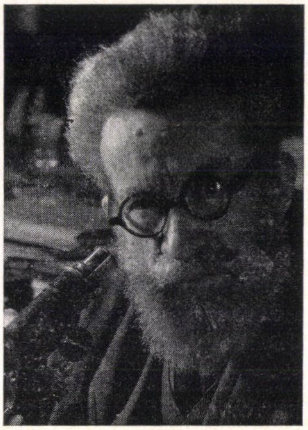
CHRONICA



ÁDÁM BOROS

1900—1973

Ádám Boros, the great scientist of Hungarian flowering plants and mosses was born on 19th November, 1900 in Budapest. He was raised from his early childhood in a natural scientific view, amidst lovers of nature and art, as his father, grandfather and uncle were equally well known teachers in a secondary school of Budapest. He soon called his teachers' attention to himself and as a result, at the age of fifteen, got acquainted with Sándor Jávorka, the world-famous author of the *Iconographia Florae Hungaricae*, then with Árpád Degen, the botanist of European fame, the author of *Flora Velebitica*, a former physician. The well-known scientists made a deep impression on the young student.

In 1918 he was admitted to the Pázmány Péter University (today: Eötvös Lóránd University), and four years later graduated and took a Ph. D. in botany, geology and paleontology. He was — even as an undergraduate — assistant to Zoltán Szabó, professor of economic botany and genetics, then to Oszkár Varga, technical university professor.

In 1922 he entered service at the Experiment Station of Medicinal Plants, as a specialist with the lowest-grade state salary. In appreciation of his bryological research work he became the Central-European referee of the "Revue Bryologique" as early as the age of 29, and fulfilled this task up to his death thereby establishing important international relations. He was appointed private docent in the subject of agricultural botany, then later in medicinal and chemical botany too. His qualification was acknowledged in 1948 too, and he was awarded the title of university professor.

From 1938 he temporarily worked at the Seed Testing Institute, and besides his bryological research work dealt with the biological questions of weeds, studied fens and caves and excelled in agrobotanical activity. From 1944 he was for three years the teacher of botany at the University of Veterinary Sciences, Budapest. Over 12 years from 1945, during the difficult postwar times he reorganized — as director of the Research Institute of Medicinal Plants — the Hungarian research work on medicinal plants and gained distinction in exploring the therapeutically important species of the Carpathian-Basin, in the botanical identification of Hungarian drugs sought for export, and even took part in elaborating medicinal plant standards up to the end of his life.

In his papers written on medicinal plants the following plants were discussed: mint, shield-fern, wild chamomile, hellebore, mullein, thyme, buck-bean, cumin, primrose, henbane, mistletoe, sweet sorghum, soap-root, sumac, digitalis, catnip, sweet-root, centaury, wild saffron, sweet-flag, belladonna, ergot, chervil, dill, etc. His monographs written in the serie of "Magyarország Kulturflórája" (Cultivated Plants of Hungary) deal mostly with medicinal plants too: *Anthriscus cerefolium*, *Crocus sativus*, *Lavandula angustifolia*, *Anethum graveolens*, *Verbascum phlomoides*, but some cultivated plants important from different aspects — like *Spergula arvensis* and *Phacelia tanacetifolia* — are discussed as well.

With his paper "Magyarország Mohaföldrajza" (Bryogeography of Hungary) he was awarded the title of academic doctor of biological sciences in 1957, then — as a continuation of this work — the principal work of his life: "Bryogeographie and Bryoflora Ungarns" was published in 1968 by the Publishing House of the Academy of Sciences.

In appreciation of his international activity and results he was elected in 1966 honorary member of the British Bryological Society (London).

Boros achieved more and more results in the field of agricultural botany too, especially when he left the Institute of Medicinal Plants and began to work at the Institute of Agrobotany, Tápiószele. As foundation member and department leader of the Institute he organized the work of collecting and evaluating the Hungarian wild species of feed value. He was an excellent floristic expert with extraordinary plant- and local knowledge. He was unselfish in imparting his knowledge and taught and helped his colleagues whole-heartedly. When retired he continued from 1960 the agrobotanical research work as an expert, but spent most of his time in working up his bryological research. He was in close connection not only with the research workers of the neighbouring countries but through the Société d'Échanges de Muscinées with the most famous bryologists of the world too. Of his recent works he did not live to see the publication of the "Bryospora Atlasz" (Bryospore Atlas) and could not complete his book on the bryoflora of the Carpathian Mountains, because on 2nd January. 1973, after a short suffering he died.

His private collection of plants and mosses containing 130 thousand samples of mosses and 65 thousand sheets of flowering plants is a national treasure protected by the State. The number of his scientific and popular publications is 731 ! His rich life-work included-besides the bryology and floristics — the study of fens and caves, researches of medicinal and fodder plants, the biology of weeds, agrobotany, the history of botany and even nature preservation.

He was primarily interested in the flora of the Carpathian Mountains and -Basin, but dealt with the flora of the mountains of Vértes, Gerecse, Velence and Bakony too, studied intensively the plants of County Somogy and of the plain of the river Drava, and in the Great Plain the flora of Nyírség and Trans-Tisza. Outside Hungary he made study tours in Austria, Czechoslovakia (XII. International Conference on Plant Geology) and in France (Pyrenees). His last collecting tours were made in 1972 in Transylvania and Northern Hungary.

His wife, Julia Kenyeres was his faithful companion in his study tours and assistant in his scientific work.

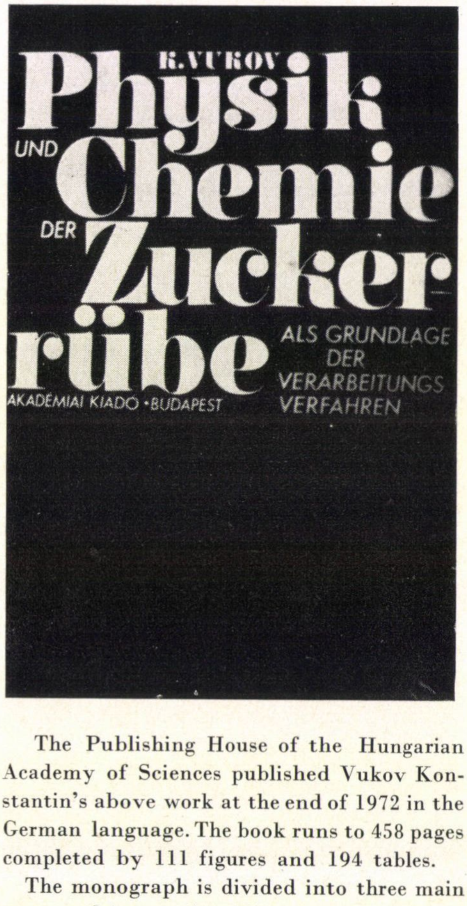
He dedicated his life to science and education. His books, scientific papers and his world-famous collection will continue to help the researchers of botany, agricultural sciences and nature protection in their work, and his honourable personality will go on living in our memory.

L. Gy. Szabó

Acta Agronomica Academiae Scientiarum Hungaricae, Tomus 24 (1—2), pp. 237—249 (1975)

RECENSIONES

K. Vukov: *Physik und Chemie der Zuckerrübe als Grundlage der Verarbeitungsverfahren.* Akadémiai Kiadó, Budapest, 1972.



K.VUKOV
Physik
UND Chemie
DER Zucker
rübe
ALS GRUNDLAGE
DER
VERARBEITUNGS
VERFAHREN
AKADÉMIAI KIADO •BUDAPEST

The Publishing House of the Hungarian Academy of Sciences published Vukov Konstantin's above work at the end of 1972 in the German language. The book runs to 458 pages completed by 111 figures and 194 tables.

The monograph is divided into three main parts and is completed by an appendix. The author discusses the following subjects: 1. Physical and chemical characteristics of sugar-beet; 2. Effects of various factors on the physical and chemical characteristics of sugar-beet; 3. The operative procedure and the physical and chemical characteristics of sugar-beet; 4. Description of some less known methods of investigation.

In the first part the knowledge of the morphological, histological and mechanical characteristics of sugar-beet is excellently summarized. It is in this part that the macroscopic structure of the sugar-beet and within this the importance of its fibre-, dry matter- and water content and the related physical and technological characteristics are discussed. The main part of the chapter — due to its importance — is the chemical composition of the sugar-beet. The role and importance of saccharose content and other sugars, ash- and electrolite content, nitrogeneous compounds, macromolecular and colloid disperse substances in the sugar technology are clarified in their details and relations. At the end of the chapter the standard characteristics of the sugar-beet are summed up, and their connection with the individual production processes disclosed. These selected standard characteristics will be discussed again in the subsequent chapters of the book in connection with some factors acting on them. The author incorporates the results of many years of his internationally highly appreciated research work in the appropriate parts of the chapter, whereby it becomes especially valuable.

The second part is a unique and excellent undertaking. As for its content, details and extension no work analysing the factors acting on the physical and chemical characteristics of sugar-beet with similar intensity can be found in the literature of sugar-beet. Of the genetic factors the effect of variety on the technological characteristics is first studied. In this context important technological parameters (cutting resistance, elasticity module, diffusion constant) to which little attention has been paid by breeders so far, although by their introduction the technological value of varieties could be further improved, are analysed. All major European sugar-beet varieties are dealt with and evaluated in relation to the individual technological parameters — even in a varietal and climatic distribution where possible. The chapter subsequently discussed the effects and correlations of climate, soil and ecological factors. The world production of sugar-beet is divided into climatic zones, and the effect of the latter on the quantity of sugar-beet produced there is analysed. The effects of growth factors and various diseases on the quality of sugar-beet are described in detail. After that the effect on the quality of production methods and techniques is dealt with, with emphasis laid on the most important of them (fertilization, irrigation, weed control, plant stand, etc.).

It is not only the growth and development of sugar-beet that determines its quality and technological value; harvesting and storage also play an important role in this respect. In the second chapter the author discusses these effects with due regard to their importance incorporating the major results of Hungarian researches too. At the end of the chapter the effects of the different factors on the quantitive features of sugar-beet are summarized in a table giving a clear picture of them. In the chapter the author works up a vast experimental and literary material including his own results as well. The treatment of the material is new both in content and in form. These extensive and practically highly important subjects are treated, summarized and synthetized by starting from the experiences, results and experimental data of sugar-beet production all over the world. In this form and using such a large experimental material this subject has not been dealt with by anybody but the author. The vast material is arranged in a clear system; new correlations are sought for and established. When processing the experimental data the author endeavours to underline the characteristic features and find the correlations.

The third part of the monograph discusses the optimalization of technological operations by starting from the properties of raw materials. Within this the individual technological operations, like the preparation and cutting of sugar-beets, extraction, clearing and distillation of the juice, crystallization of sugar and separation of crystals, are dealt with in detail. Factors acting in the course of the technological operations as well as their interactions are analysed. Each part is completed by a rich factual material.

The description of the so called less known methods, the discussion of their application possibilities, advantages and deficiencies especially promote the work of practical experts.

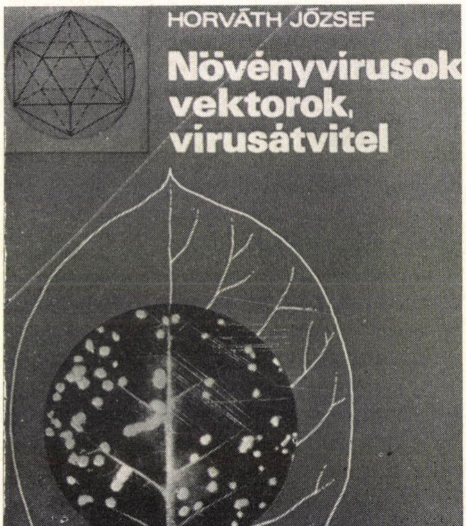
Vukov Konstantin's work supplies a great want in the literature of the sugar-beet and sugar industry. No similar work has been known so far. The physical and chemical properties of the sugar-beet, the effects of various factors and the optimalization of technological processes are treated in a new, manyfold and comprehensive way. The individual elements of complicated interactions are analysed one by one, then synthetized. Synthesis is one of author's main endeavours which starts, however, from the most meticulous presentation of details. A vast literary material is processed, critically evaluated and incorporated in the author's work.

Nevertheless, the monograph is by no means a mere literary compilation since it is based first of all on the author's own research results. His results — even the most recent ones — of many years spent in an internationally outstanding research work are integral parts of his book. Biological and technological views and knowledge are efficiently combined in each part of the work.

The monograph can be used as a manual by the experts of sugar industry, sugar-beet production and breeding all over the world. Through the rich research material, the new results and method found in it this book is also a source of further researches made in the field of production and the sugar industry. The publication of the monograph was preceded by great international interest. Its success was characterized by the fact that the German edition had hardly been published when the author began to compile an enlarged English manuscript.

L. Magassy

J. Horváth: *Plant viruses, vectors, virus transmission.* Akadémiai Kiadó, Budapest, 1972.



The handbook discussing the properties of plant viruses and mycoplasms, the ways of transmission, the biological importance of the vectors involved in transmission and the most recent testing methods supplies a long felt need and, though published in the Hungarian language, is made useful for foreign readers too by its tables and international symbols. Being written by a research worker internationally recognized in the field of experimental plant virology it deals with the recent tendencies of the subject on a sound theoretical basis, discussing the results of the last years in full detail and presenting the data of the numerous studies in a clear tabular form. With its exact terminology of natural science and by a synthesis of the divergent data the book serves the purposes of applied biology first of all in agricultural and horticultural relations.

The handbook published on 515 pages by the Publishing House of the Hungarian Academy of Sciences is divided into eleven chapters and completed with 89 text figures and references to 1882 bibliographic data. The use of the book is greatly facilitated by an English list and cryptograms of pathogenous plant viruses, which is an arrangement unprecedented even in the world literature. The symbols used ensure a clear understanding. With the definition and brief explanation of the terminology used the book offers great help to those interested in the results attained in the scope of this subject; the solution is very successful with respect to both content and form. The index of this handbook, which while encompassing a wide range of data is extremely concise, compiled on the basis of modern principles, is also very useful.

The book begins with the preface of J. Szirmai, the well known founder of Hungarian plant virology, then, after the author's introduction follows a short survey of science history, with an evaluation of the outstanding results of plant virology and mycoplasmology from the point of view of molecular genetics.

The chapter discussing in detail the general characteristics of pathogenous plant viruses is of special importance for experimental biology. The presentation of the chemical composition an ultrastructure of plant viruses reflects the most recent views and cover literature on the subject up to the end of 1971. Especially detailed information is given by the author on the chemical composition (5.6 per cent ribonucleic acid and 94.6 per cent protein content) of the model virus (Tobacco mosaic virus), the micromorphology and central position of the double spiral ribonucleic acid, as well as on the screwlike arrangement of the protein subunits of 17—18 thousand molecular weight. The author emphasizes that the mycoplasms — unlike the plant viruses — always contain deoxy ribonucleic acid (1.5—4 per cent) and a cell structure analogous with the central position ribosome in addition to ribonucleic acid (3—10 per cent). The handbook points out that the most important structural element of the plant virus is the virulent ribonucleic acid (virion) which is covered by the definite structures of the protein subunits. On the other hand, the deoxy ribonucleic acid content of the plant mycoplasm stores genetic information and the latter controls a "cell work" maintaining the biosynthetic processes and structure too. The book reveals, further, that the plant virus does not posses any metabolism, and so is not able to synthesize the organic compounds of energy content, but relies completely on the metabolism of the host cell. At the same time, on synthetic culture media the mycoplasm has the capacity of reproduction, which is connected with its controlled metabolism.

The serology of viruses is a highly important field of experimental plant virology; it is excellently suitable for identifying the viruses and studying the details of infection processes. The principle of experimental testing is also given in this chapter, namely: under the influence of the antigen (plant virus) introduced into the animal organisms an antibody is produced in the blood serum, and the two together can easily be compared in the antiserum experimentally with the control, as normal serum. This way the effect induced by the purified plant virus isolate can be serologically well characterized by means of precipitation and agglutination reactions as well as with complement binding and anaphylaxy tests. The detailed description of the tests and methods together with a summary of the literary material recommended gives a substantial help to those interested in the subject.

The author strictly distinguishes plant virus inoculation from virus infection, since the penetration of the virus or virus ribonucleic acid into the living cell can be regarded as inoculation through the cells injuries, while the actual infection occur in the place or centre of infection, and the number of the latter is usually much less. From a virological point of view, the sieve-tubes are of outstanding importance among the phloem elements. The sieve-tubes which do not contain cell nuclei are in functional connection with each other through fine threads. The author points out that an especially vigorous virus reproduction can be observed on definite spots of the latter (in the ectodesms) irrespective of the fact that the plasmodesmic connection of the sieve-tubes provides a direct possibility for the plant viruses to spread inside the host organism.

The reproduction of the plant virus, which is a synthesis independent of deoxy ribonucleic acid and dependent of ribonucleic acid, is described by the author keeping in view the factual data of molecular genetics. The double spiral ribonucleic acid produced is enveloped by the subunits of protein. On this basis the ultrastructure of the viruses follows from the symmetry of the virion, which is discussed in detail in the chapter. The author summarizes the virological results of the last years in a synthetizing way and his conclusions are characterized by thoughtful moderateness.

Symptomatology is a special field studied by the author with extreme thoroughness; he distinguishes external syndromes visible to the naked eye, and microscopically evaluable internal syndromes. Besides the electron microscopic micromorphology this chapter of the handbook is of special value, since in the field of symptoms presented in the interaction of oversensitivity reactions related with the virus infection new conceptions have developed mostly on the basis of the author's original experimental results. This chapter is highly remarkable if only because it sharply distinguishes the symptoms of plant virus infection from other disease symptoms, and marks them with internationally used indices as symbols. In the indications of the symptoms three principles are dominant: 1. infected plant parts are indicated with capital letters, 2. disease symptoms with small letters, and 3. pathological characters with special symbols. The developed system of symbols renders the book easy to survey and homogeneous from the point of view of special literature.

A separate chapter deals with the results attained in the field of plant virus control, and considers the possibilities of protection first of all from the point of view of practical application. It gives an account of the results of applying adenine, uracil, benzimidazole derivatives and of those attained with antibiotics and discusses the possibilities of using other special inhibitors from the aspects of prevention and therapy separately.

The handbook describes in detail and in a didactic distribution the ways pathogeneous plant viruses are transmitted (by grafting, mechanically, through insect vectors, etc.) as well as the role and virological importance of factors influencing the relationship between virus and host plant (temperature, light, chemical compounds, biotic and abiotic factors), first of all from a practical point of view. Special emphasis is laid on the possibilities and applicability of heat inactivation.

A separate chapter which sums up the significance of vectors in practical plant protection discusses in detail the extreme importance of basic experiments. It gives, at the same time, the test results of international vector-host investigations in tables. On the basis of experimental results the author proves that the organs of host plants serve as sources of infection in different degrees in the case of different vectors, at the same time the age and physiological state of the vectors also influence the spread of the plant viruses.

The usefulness of the handbook is greatly increased by the carefully arranged tables which show the virus transmission methods of various vectors. A great advantage of the book is, further, the scientific and English lists of vectors and viruses which provide the possibility of even foreign readers using the tables. The chapter presents the aphid vectors of about 300 pathogenous plant viruses with the host organisms indicated. Beside the aphids virus transmission by the cicadas is the most important; in the organs of the latter plant virus infection is also different as demonstrated by the experimental data.

Research work carried out recently has revealed that the 34 mycoplasms identified occur on a much higher number of host plants, and 10 specific insect vectors are known so far.

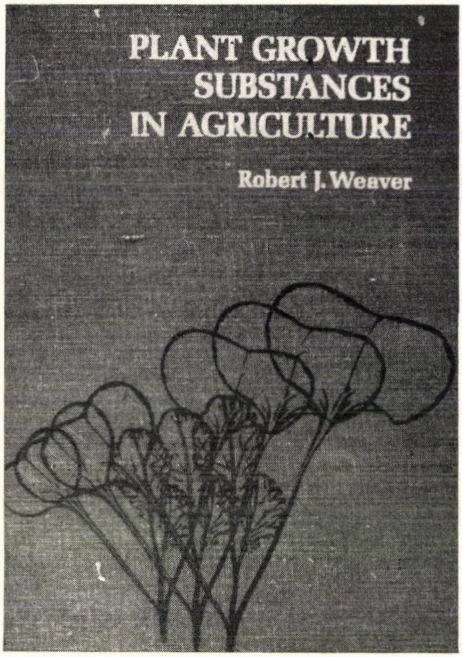
A great value of the handbook is its discussing the role of mandibular insect vectors as well as virus transmission by nematodes and sucking insects. From a practical point of view the wide range of data collected by the author are also remarkable; they demonstrate that virus transmission by *Cuscuta* could be proved in 71 cases, the vector role of lower fungi in 9, transmission by seeds in 49 and spreading by pollen in 12 cases.

The author, a highly competent expert in plant virology and mycoplasmatology with his exact formulation, with the synthesis and systematization of data pertaining to the subject as well as with the detailed discussion and tabulated presentation of the methodology of virus transmission offers helpful assistance not only to those interested in the subject but also the specialists working in related fields of science.

B. I. POZSÁR

R. J. WEAVER: *Plant growth substances in agriculture.* W. H. Freeman and Company, San Francisco, 1972, 594 p.

Chemization in agriculture is increasing at a rapid rate these days; every now and then new chemicals are introduced in agri-


PLANT GROWTH SUBSTANCES IN AGRICULTURE
Robert J. Weaver

culture to attain objectives useful for man. In spite of the results obtained so far the possibilities offered by plant physiological research have not been yet fully utilized in the agricultural practice. This is clearly seen in Robert J. Weaver's book "Plant growth substances in agriculture" published in 1972, which for its timeliness and intention of encouraging is welcome.

The author is a professor at the Institute of Viticulture, University of California (Davis) who has been dealing with plant growth substances and their practical application since 1944 and is thus an excellent specialist of this subject. From 1948 his research work has mostly been related with the endogenous and exogenous growth regulators of grapes but he follows with attention the results of researches carried on with other crops too. He has published more than 100 papers on the subject of plant growth substances.

All growth and development processes of the plants are under the control of chemical regulators. Phytohormones naturally occurring in the plants and many exogenous organic compounds are able to control these processes at very low concentrations. In most plants the developmental phenomena can be advantageously altered by human interference. "It is quite possible, that, in time, physiological processes in plants will be controlled by application of growth substances" — writes the author in the preface of his book.

The book is written largely from an agricultural point of view and stresses both present and possible future important commercial uses of growth substances in agriculture. However, in the first four of the 12 chapters of the book the author — quite rightly — gives theoretical information necessary for understanding the problems of practical application.

Chapter 1 deals with the nomenclature and the historical aspects of the growth substances. Indeed, the clarification of the nomenclature is an important task since the authors often use the same term in a different sense, and group the large number of growth substances in a different way. Weaver's nomenclature is reasonable and logical. He is not, however, sufficiently consequent in grouping the growth substances inasmuch as he sometimes regards ethylene as a hormone and discusses it among the phytohormones while another time omits it from their list. On the other hand, the group of inhibitors is rather heterogeneous, and it is a question whether e.g. the phenolic compounds, the benzoic acid and cinnamic acid derivatives which either inhibit or stimulate the growth depending on the concentration can unanimously be regarded as inhibitors.

The biological and chemical determination methods of growth substances are described in Chapter 2. The author discusses the extraction and purification techniques of auxins, gibberellins, cytokinins and inhibitors, and besides the traditional bioassays describes the most up-to-date chemical and instrumental determination methods used at present. The demonstration and identification methods of ethylene, however, have unfortunately been left out of the chapter. The detailed description and schematic representation of various bioassays are suitable for showing the university students the way to reproduce them in the plant physiological exercises.

Chapter 3 discusses the occurrence and chemical nature of growth substances in all five groups of regulators (auxins, gibberellins, citokinins, inhibitors and ethylene). Besides the chemical structure and chemical designation the book — wherever possible — gives the trivial and trade names of growth substances too.

Chapter 4 gives information on the highly diversified biological effects and action mechanism of growth substances. The breadth of the action spectra itself indicates the wide applicability of these regulators. The essence of the action mechanism of plant hormones in the author's opinion is a cellular enzyme regulation taking place through the influence they exercise on the nucleic acid metabolism. This opinion agrees with the present view of genetics that in all probability the hormones act on a molecular or gene level in the cells. The author points to the importance of the interaction and relative quantitative ratio of the hormones, as well as to the role the external environmental factors play in modifying their effect.

After this sound theoretical foundation the following eight chapters give information on the different ways and possibilities of utilizing the growth substances in horticulture, viticulture, forestry, glasshouse and outdoor crop production and plant breeding. This practical part of the book is a full and systematic summary of the available literature, in fact a large-scale review. Accordingly, on each occasion of describing a practical application the author refers to publications by authentic authors. When processing the large number of available literary data, the proper systematization must have required much work and caused great concern, since the material can be classified from different aspects. The difficulties are increased by the fact that certain subjects are closely related or even overlap each other, and so can be discussed in more than one chapter. R. J. Weaver has solved most of these systematization problems with success, so — in spite of the manyfoldedness of the subject —the structure of his book is clear-cut. The only deficiency of the pratical part is that the author does not make comments when presenting the literary publications.

The problems of rooting and propagation are summed up in Chapter 5. First a theoretical information is given on the anatomical and physiological bases of root formation, and on the factors and cofactors required for the root formation. This is followed by a discussion on the practical utilization of growth substances promoting the root formation of cuttings, and on the applicable methods and concentrations, according to the special demands of various plants and plant groups.

Chapter 6 deals with the dormancy of seeds and buds. The causes and physiological peculiarities of dormancy, as well as the role of the phytohormones in the onset and termination of rest are surveyed. The artificial breaking of dormancy in not readily germinating seeds of forest trees and fruit trees, in buds of woody plants and in certain tubers and bulbs — that is, the stimulation of germination or sprouting — is described. But a satisfactory information is also given on the maintenance of dormancy in these organs, that is, on the possibilites of prolonging the sprouting. The description of methods aimed at delaying sprouting in plant parts (potato, onion, beets, etc.) stored for nutrition purposes is particularly noteworthy.

In the part dealing with flowering (Chapter 7) the author clarifies first of all the physiological aspects of floral initiation (vernalization, photoperiodism), as well as the importance of various regulators in these processes. We are then acquainted with various possibilities of inducing and accelerating, as well as preventing and delaying flowering in a number of plants by chemical interference. Special emphasis is laid on the stimulation of flowering in fruit trees, tomato and ornamental plants. The author finally describes the method of controlling the sex expression, that is, increasing the number of female flowers with the view of attaining higher yields.

In accordance with their great practical importance, fruit set and development are discussed in full detail (Chapter 8). Here too, the author gives a theoretical foundation by summarizing the physiology of pollination, fertilization and fruit development. The chapter then describes the current and potential methods of utilizing various growth substances in controlling the fruit development. It reveals the possibilities of accelerating the ripening of various fruits (apple, pear, grape and other berries, tomato, bean, etc.), increasing the fruit size and preventing the frost injury of flowers and fruits. Successful methods are further described to delay fruit ripening in certain cases.

The subject of Chapter 9 is senescence. After a discussion on the physiological processes characteristic of senescence we are acquainted with the role of externally used regulators in controlling the process of senescence. Reference is made to the senescence of excized leaves and detached shoots, which, however, is a mostly theoretical problem. After this the author deals with a number of practical questions mainly of interest to horticulturists. He explains how the senescence of vegetables during storage can be delayed by the application of growth regulators (mainly cytokinins), and how the senescence of stored fruits can be prolonged with the aim of preventing damages caused by overripening.

Chapter 10, dealing with the subject of abscission, is also very interesting. The author again provides a theoretical basis by informing the reader about the anatomical causes of leaf abscission and the hormonal control of leaf and fruit drop, then gives a detailed description of the methods of controlling leaf abscission in agricultural crops. There are excellent methods e.g. of facilitating the harvest of cotton, bean, woody plants by defoliation; of delaying abscission in the case of cut flowers and ornamentals; methods for preventing the premature falling of young and ripe fruits, and even of inducing fruit drop.

Chapter 11: "Size control and related phenomena" is rather heterogeneous, some of its parts could be discussed in other chapters as well. The following subjects are included here: growth stimulation of grasses, increase of sugar production in sugar cane, yield increase in cereals and legumes, chemical influence exercised on the crotch-angle in fruit trees and ornamentals, growth inhibition of various plants, prevention of lodging in cereals, promotion of drought, salt and cold tolerance in various plants, and finally, chemical enhancement of resistance to diseases and insects.

In accordance with the high practical importance of the subject it deals with, Chapter 12 discusses the question of weed control at great length. It is essential to mention here that of the large number of weed killers used in the practice only the auxin-type herbicides are dealt with by the author. In the introduction the importance, history, biological effect and action mechanism of herbicides, as well as the question of selectivity are spoken of. Practical application unconditionally demands the description of herbicide uptake and translocation. After this 28 hormone-like herbicides are presented, divided into three groups: chlorophenoxy compounds, chlorobenzoic acids, and other auxin-type herbicides. The author gives information about the practical application of all presented weed killers: what plants, how, when and at what concentrations can be treated with them.

In Chapters 5—12 the description of the agricultural utilization methods of growth substances can thus be considered complete. As an only deficiency we may mention the lack of references to whether the chemical treatments of plant parts destined for human or animal consumption has any effect on the palatableness of the product or the health of the consumer.

All chapters dealing with practical aspects make it clear — what the author emphasizes in the preface anyway — that no advice of general validity can be given in influencing developmental processes with definite purposes, since the results vary according to the species and age of the plants, the quality and concentration of the chemicals applied and the method of application. Therefore, the growers have to consult with experts before using the regulators. The local climatic and soil conditions may also modify the result. Further, it must be taken in consideration that the same result can be attained with different growth substances and in more than one way. Owing to these circumstances it is advisable to determine the method of application experimentally.

R. J. Weaver's book is an excellent guide for agricultural experts, but it may be of use in higher education as well. It can especially be recommended as a material of special courses at agricultural and horticultural universities, since in the framework of the plant physiological lectures no opportunity is given to treat the practical aspects of growth regulation at full length.

The documentation of the book is ample and high level. The figures and photos are of very good quality. The language and structure of the text is clear and easy to understand. The getting up is aesthetic.

Each chapter is followed by numerous literary references relevant to the subject. A selected list of the cited publications— some 1100 up-to-date works — can be found at the end of the book.

The publication of R. J. Weaver's book remedies a deficiency in the special literature of these days, and encourages the readers to the widest possible introduction and utilization of the recommended practical methods.

M. VARGA

REVISTA CUBANA DE CIENCIAS VETERINARIAS, 1971, *2*, 1.

The Revista Cubana de Ciencias Veterinarias, the official gazette of the Organo del Consejo Cientifico Veterinario, organization of the Cuban People's Republic engaged in teaching veterinarian sciences, in No. 1. of vol. 2. published in June 1971 inserted the following articles:

1. O. N. RODRIGUEZ, A. RIVAS: *Estudios hematológicos en la Babesiosis y Anaplamosis* (Hematological study on cattle Babesiosis and Anaplasmosis) delivered on behalf of the Havana Veterinary School at the XIX. World Congress of Veterinarians and Animal Breeders organized in Mexico, August 1971.

REPUBLICA DE CUBA

revista cubana de
ciencias
veterinarias

VOLUMEN 2 — NUMERO 1
ENERO - JUNIO 1971

órgano del
Consejo Científico Veterinario

The authors examined over three and a half years 315 cattle, 198 of them (103 Holstein, 67 Jersey, 28 zebus) after natural and 117 (82 Holstein, 35 Jersey) after artificial infection. Clotting of blood samples required for the examinations was inhibited with Ethylene-diamine-tetra sodium acetate which — as a bactericide — being at the same time a preservative too, solved the difficulties of storing and cooling the samples. Bone-marrow, spleen and lymphatic glands were studied by means of biopsia. For the analyses the Giemsa- and Pappenheim-staining methods, methyl-pyronin, brillent cressyl-blue, Sudan black colouring agent, para-amino-salicylic acid were used by means of histochemical procedures alkalic phosphatase, peroxidase, non-specific esterase enzimes were demonstrated.

Samples were prepared for the analyses

in the form of blood smears and impressions; in the course of the analyses methods based on phase contrast and light interference were employed. During the examinations poikilocytosis leucocytosis, anisocytosis, lymphocytosis, monocytosis, reticulocytosis and erythroblastaemia were found. In the hyperplasic bone-marrow plasm cells and macrophages were pointed out. The pathogens proved to be *Babesia bigemina, argentina, Anaplasma marginale.* The examinations are considered successful because in this way the veterinarian can conclude on the result of the treatment not only from the clinical symptoms but also from changes in the blood picture, further, besides the classical examinations the modern histochemistry was applied too.

2. J. Mitat: *Estado actual de las investigaciones de los marcadores genéticos y sus aplicaciones zootécnicas en Cuba* (Actual study on the genetic characters and their application in the livestock-breeding in Cuba) delivered by the author, a research worker of the Immunogenetic Laboratory of the Cattle Breeding Faculty of Havana University, at the VI. Pan-American Congress of Veterinarians and Animal Breeders organized in Santiago, Chile, in September 1970.

3. R. Ronda, J. Pilz, J. Mitat: *Frecuencias génicas de cinco loci de grupo sanguinev en la raza Holstein-Friesian en Cuba* (Frequency of occurrence of five blood groups in the Cuban Holstein-Friesian crossings).

18. R. Stanek, J. Mitat, Lilia Ezcurra: *Estudio electroforético del polimorfismo proteico en razas bovinas criadas en Cuba.* Polimorfismo de las transferrinas y amilasas (Electroforetic study on the polymorphism of proteins in cattle breeds. Polymorphism of transferrines and amylases).

Every country which wants to develop its livestock farming introduces the immunogenetic examinations which is especially important in the progeny control, to prevent the narrowing of animal line in the case of artificial insemination.

4. Maria E. Torano: *Estudio de la dinamica de los minerales osteotroficos en el suero de la sangre de terneros de raza Holstein* (Study on the dynamics of osteotrophic minerals in the blood serum of Holstein calves).

An important feature of the article is that the results of examinations published in it were evaluated by means of biometry, but at the same time the principle of alkali earth alkalinity developed by Marek—Wellmann—Urbányi which cannot be neglected in bone examinations and mineral circulation studies was left out of consideration.

R. Brito J. Castellanos, Y. Rizo, J. W. Gonzáles, M. Hernández, C. Iglesias, J. Gnerra: *Aportaciones sobre investigation de enfermedades que afecten el aparato genital de las hembras bovinas en Cuba* (Financial support to investigations into the sterility of cattle in Cuba). A lecture delivered by the authors — research workers of the Veterinary and Livestock Breeding Faculty, Physiological and Pathological Institute as well as Veterinary School of the Havana University, and of the National Veterinary Institute — at the IV. Pan-American Congress of Veterinarians and Animal Breeders in Santiago, Chile, 1970.

On the instructions of the Revolutionary Government sterility studies were performed with 1 million cattle of which 53517, that is 5.4 per cent, were found sterile.

Sterility could be traced back in 54.28 per cent of the cases (29049 animals) to functional causes, in 43 per cent (23012) to infectious diseases, in 1.05 per cent (562) to other detected, while in 1.67 per cent (894) to unknown causes. For the purpose of treatment application of vitamin A, iodine, phosphorus and manganese preparations is recommended.

It should be noted that sterility requires not only treatment but also prevention and that in such a way as reproducing individuals which, in spite of the inadequate feeding and keeping methods, are fertile and show a relative productivity even under adverse conditions.

6. L. Vrzgula, Maria E. Torano: *Perturbaciones electroliticas en el snero de terneros afectados por diarrea.* Rehidratacion como parte importante de la terapia (Disturbances in the blood electrolyte balance of artificially

raised calves affected by diarrhoea. Rehydratation as an important part of therapy).

The article deals with the diarrhoea of artificially raised calves, with its complication: the exsiccosis, and its treatment. For the purposes of treatments various physiological solutions are recommended, e.g.: Ringer, Sherry-Grinyer, Mills, Darrow, sugar solution and amino acid solution prepared from hydrolysed casein.

With the diarrhoea of calves again, greater emphasis should be laid upon prevention, which can be achieved on the basis of the principle developed by Nyiredy, namely: milk given to the calves must be of the same quality — in every respect but especially microbiologically — as that given to infants.

7. A. Delgado, R. Prieto: *Eficacia antihelmintica de la combinaction de Tetramisole Niclosamida en rumiantes* (Antihelmintic effect of the combination of Tetramisole and Niclosamide in ruminants).

The most recent and most efficient, less toxic internal antiparasitic preparations: Niclosamide (2-chloro-4'-nitro-5 chloro-salicyl-amide) and Tetramisole (dl-2,3,5,6-tetrahydro-6-phenyl-imidazo[2.1-b]thiazol) were tried out by the authors in 88 Holstein and 83 Holstein × zebu F_2 calves of 3—5 months of age and 70—110 kg weight.

Of Niclosamide 1 g and of Tetramisole 6 g were applied. The same preparations were tried out in 27 4—5 months old Persian lambs of 19—30 kg weight. Of Tetramisole 0.5 and of Niclosamide 3 g were applied.

When applied jointly the two products — which in agreement with the Hungarian researchers' investigations proved highly efficient — were successful in controlling *Monieza*, *Dictyocaulus*, *Haemonchus*, *Cooperia*, *Trichostrongylus*, *Trichuris* and *Strongyloides* species. The results of treatments were controlled with coprological tests.

8. D. Ovies, V. Jurásek, V. Tokarev: *Evaluacion de la actividad del Tetramisole y del Adipato de Piperazina contra la ascaridiosis de las gallinas.* (Evaluation of the effect of Tetramisole and Piperazine-adipate on ascaridiosis in poultry). Lecture delivered by the authors at the XIX. Congress of Veterinarians and Animal Breeders organized in Mexico. The authors tried out the effect of Tetramisole and Piperazine-adipate against *Ascaridia galli* with 30 Leghorn poultry. Of Tetramisole 25 and 40, of Piperazine-adipate 300 mg/kg were applied.

When applied in the above dose Piperazine-adipate destroys 50 per cent of the larvae. 59.09 per cent of the immature insects and 99.9 per cent of the adults.

The effect of Tetramisole when applied in a 40 mg/kg dose was found hundred per cent.

In the second experiment carried out with 255 poultry of Leghorn breed Tetramisole applied at a dose of 40 mg/kg was found to be of 87.1, while 300 mg/kg Piperazine-adipate of 70.6 per cent efficiency.

In the case of invasion causing repeated infections Tetramisole application yielded 94.6 and Piperazine-adipate 63.4 per cent results.

9. V. Jurásek, D. Ovies, L. Espaine: *Primer hallazgo del trematodo Brachylaemus suis Balozet, 1936 (Brachylaemidae) en los cerdos de Cuba.* (First case of observing Brachylaemus suis Balozet, 1936 *(Brachylaemidae)* in Cuba swines). This rare parazite was first described by Balozet in 1936, Tunis. Rare pathological phenomena, too must be followed with attention even if they have no economic importance for the time being, because one never knows when the ecological and pathological conditions will be favourable to their spreading.

10. M. Oplistil, Maria E. Torano y V. Dycková: *Metabolismo ácidobásico en los animales.* III. Determinación del pH actual de la sangre por el micrométodo de Astrup (Acid-base metabolism in animals. III. Determination of pH in blood by Astrup's micromethod).

It is a worldwide and at the same time necessary phenomenon that experts working far from laboratories make themselves more and more independent of the central laboratories, perform the examination on the spot and with the possibility of a rapid intervention obtain results as soon as possible. The method described in the article serves this purpose too.

11. R. **Prieto**: *Eficacia de la Niclosamide en el tratamiento de la monieziosis en bovinos y ovinos jovenes* (Effect of Niclosamide treatment of monieziosis in cattle and young sheep).

Niclosamide was applied at a dose of 65–72 mg/kg to young cattle, and at 70–90 mg/kg to young sheep against infection by *Moniezia benedeni* and *Moniezia expensa.*

A 40-fold overdose of the therapic dose was not found toxic. 72 hours after the treatment the coprological tests showed negative results.

12. P. **Metev**, A. **Benitez**: *Tipificación y antibiograma de cepas de Salmonellas aisladas de visceras de terneros muertos por salmonelosis* (Typification of *Salmonella* strains bred from the viscera of calves died of salmonellosis, and determination of their antibiograms). The authors isolated 35 *Salmonella* strains from dead calves. 66.6 per cent of the strains was found to be S. dublin, and 33.4 per cent S. enteritidis.

86 per cent of the strains proved highly sensitive to tetracycline and 71.4 per cent to furodone. To nitrofurane, polymyxine B and chloramphenicole the strains displayed but a moderate sensitivity. The publication gives an assessment made on the basis of routine tests.

13. H. **Fernández**, P. **Chavez**, R. **Polanco**: *Reporte de un brote de encefalomielitis equina tipo Este.* Medidas de control (Report on the development of Eastern type horse encephalitis). Lecture delivered by the authors in August 1971 at the XIX. Veterinary and Animal Breeder World Congress in Mexico. In the article the authors — in addition to giving a virus isolation- and serological report — deal with vector control and prevention too.

14. A. **Delgado**: *Evaluación de la efectividad antinematódica del Tetramisol* (Evaluation of the effect of Tetramisole against nematodes). Tetramisole was tested with 60 calves, 100 pigs and 28 chickens. With calves 100 per cent results were attained against *Dictocaulus*, *Cooperia*, *Haemonchus*, *Trichostrongylus*, and 40–45 per cent results against Strongyloides species. In the case of pigs the result was 100 per cent against *Metastrongylus* and *Ascaris*, 98 per cent against *Oesophagostomum* and *Hyostrongylus*, 90 per cent against *Strongyloides* and 73 per cent against *Trichuris* species.

The effect was 100 per cent against *Ascaridiosis* in poultry. Pigs were the most sensitive to overdosage. Dosages applied to the different groups of 3–4 months old calves of 100–120 kg weight were: 10 mg/kg in a 10 per cent solution subcutaneously, 12.5 mg/kg in a 3 per cent solution per os, 15 mg/kg combined with Fenotiazin cyanoacetic hydrazide subcutaneously. For pigs the dosage was 10 mg/kg in a 10 per cent solution subcutaneously.

For pigs dosage in Group I (20–25 kg) was 10 mg/kg in a 10 per cent solution subcutaneously, in Group II. 15 mg/kg subcutaneously.

To poultry (Leghorn) with 54 g weight 10 mg, with 107 g body weight 20 mg and with 160 g body weight 30 mg/kg was applied per os.

15. J. **de la Cruz**: *Nuevos reportes de ectoparásitos de los animales de laboratorio de Cuba* (New reports on the ectoparasites of laboratory animals in Cuba).

Parasitosis too, must be trated in laboratory animals or else the results of experiments cannot be evaluated because of death cases caused by intercurrent diseases.

16. L. **Giménez**, **Onelia Santos**, J. **Ferrer**: *Cirurgia radical en un caso de piómetra en leona* (Radical surgical intervention to cure pyometry in lion).

The only way of treating pyometry is in most cases the removal of the uterus. This is what the publication confirms too. The illustration of the operative technique by photos made of this rare case would have been very useful.

17. O. N. **Rodriguez**, V. **Jurásek**, L. **Espaine**, A. **Rivas**: *Reporte preliminar de la presencia de Theileria mutans (Theiler 1906) en el genado vasuno de Cuba).* Preliminary report on an infection caused by *Theileria mutans* (Theiler 1906) to the cattle stock in Cuba.)

By studying the Gazette we gain information on the health conditions of the livestock in Cuba. We can follow with attention the development of veterinary hygiene and animal breeding which were completely neglected before the socialist revolution. Data of the publications are known by the experts of the socialist and leading capitalist countries, confirm their findings and experimental results, and adapt them to the Cuban conditions. According to the conclusions drawn from the publications animal hygiene in Cuba has made a great progress since the revolution, and this progress will accelerate when the Cuban experts learn more from the scientific results of the great personalities of veterinary science and animal breeding: Hutyra, Marek, Wellmann, Jármay, Preiss, Hetzel, Csukás, Schandl, Fettick, Kertay, Manninger and Nyiredy.

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In Vol. 23/3—4 of our journal on page 514 line 2, for *South America. Bezostaya* read *South America, Bezostaya* and on the last line for *S. Rajk* read *S. Rajki.*

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РЕЗЮМЕ

СРАВНИТЕЛЬНОЕ ИЗУЧЕНИЕ ПЛЕМЕННОЙ ПРОИЗВОДИТЕЛЬНОСТИ РАЗНЫХ ПОРОД СВИНЕЙ ПРИ ПРОМЫШЛЕННОМ РАЗВЕДЕНИИ

Л. ЧИРЕ, П. ВЕСЕЛИ, Д. ШИМОН

На свиной ферме, оборудованной целиком для закрытого разведения, с 1969 до ноября 1970 года сравнивалась племенная производительность (число живорожденных поросят, их индивидуальный вес, а также число и вес поросят в возрасте 28 и 85 дней) пород венгерской белой мясного типа, шведской крупно-белой, голландской низменной, английской низменной, бельгийской Пиетрейн и американской Хемшир в ходе их чистопородного и гибридного разведения. Результаты опытов, с одной стороны показали, что при закрытом разведении надо иметь в виду разную чувствительность пород, с другой стороны, подтвердили и то, что на ферме такой системы с целью повышения энергии роста поросят и понижения убытков вскармливания целесообразно проводить скрещивания между породами.

СПОНТАННОЕ ДИПЛОИДИЗИРОВАНИЕ ГАПЛОИДА NICOTIANA SILVESTRIS SPEG. ET COMES

Л. СИЛАДИ

Цитологические анализы были проведены на полученных из ткани пыльника гаплоидных растениях *N. silvestris* и их семенном потомстве. Гаплоидные растения, в отличие от диплоидов, имели узкие листья и мелкие цветы. В их соматических клетках имелось по 12 хромосом, а в мейозе кроме унивалентов наблюдались только 1—2 бивалента. Тем не менее редко встречались и диплоидные клетки с 12 бивалентами. Из разных распределений анафазных хромосом по-видимому только гаметы, имеющие 12 хромосом, являются жизнеспособными. Фертильность пыльцы гаплоидных растений была относительно высокая, 16,7%, что указывает на большое количество нередуцированных пыльцевых зерен. Потомства гаплоидного *N. silvestris*, таким образом, опять имели диплоидные ($2n = 24$) наборы хромосом и правильный мейоз ($n = 12_{II}$). Вторичные ассоциации унивалентов (6 пар унивалентов), часто наблюдавшихся в метафазе мейоза I. гаплоидного *N. silvestris*, позволяют заключить, что основное число хромосом рода *Nicotiana* равно 6.

КУЛЬТИВИРОВАНИЕ ЗАВЯЗИ ВИДОВ RUBUS НА ПИТАТЕЛЬНОЙ СРЕДЕ

И. М. ЗАТЬКО, И. ШИМОН

Завязи из дутонов цветка сорта малины **Malling Promise** (*Rubus idaeus* L.) и **Rubus caesius** L. до оплодотворения были помещены и культвированы на питательной среде Миллера и Нитша и их модификациях. Завязи на питательной среде Нитша + ИАА 2 ппм стали самыми крупными, что можно приписать сильному образованию каллюса, тем не менее они не достигли по величине и окраске ягод, развивающихся в естественных условиях. Ягоды не имели жизнеспособного зародыша, значит они оказались партенокарпическими. На питательной среде Нитша + ИАА 0,1 ппм после 2 месяцев было обнаружено возникновение костянки семян. Препарированные из более крупных бутонов завязи образовывали более крупные ягоды. Завязи некоторых образцов **Robus caesius** на питательной среде Нитша + ИАА 0,1 ппм без пересадки оставались живыми в течение 1 года. Это удивительное явление обращает внимание на то, что обеспечение стерильных условий при хранении свежих ягод может иметь серьезное значение.

ИССЛЕДОВАНИЯ ПО ОПРЕДЕЛЕНИЮ ПОТРЕБНОСТИ ЛИЗИНА И МЕТИОНИНА

II. Определение обеспеченности аминокислот у откормочных свиней с помощью определения отдельных параметров кровяной плазмы

ДЬ. ЙЕЧАИ, М. СЕЛЕНИ-ГАЛАНТАИ, Б. ЮХАС

У нескольких групп свиней изучался эффект добавленных в корм аминокислот по содержанию свободных аминокислот кровяной плазмы. В течение опытов в группах по 5 животных определялся свободный аминокислотный состав плазмы из кровяных проб. Установлено, что разные дозы аминокислот, добавленные в диету, чувствительно влияют на содержание свободных аминокислот кровяной плазмы. При наличии в корме 0,92% лизина был найден оптимум уровня лизина плазмы (27—29 умол/100 мл плазмы). Результаты авторов по изучению азотооборота соответствовали вышеприведенным определениям. Их опыты показывают, что определение содержания свободных аминокислот плазмы как параметр нормы потребности свиней в аминокислотах может быть использован как точный, быстрый и чувствительный метод.

ИОНОБМЕН SCENEDESMUS OBTUSIUSCULUS

Г. МЕСЕШ

В предыдущих опытах нами показано, что механизм усвоения калия и бромида ***Scenedesmus obtusiusculus*** зависит от стадии развития клеток. Кривая характера концентрации, компетиция, стимулирующий эффект света указывают на активное усвоение главным образом калия. Зато активность механизма усвоения иона бромида является сомнительной. Результаты опытов по обмену подтверждают, что обмен бромида является скорее активным процессом, чем процессом усвоения. Свет в значительной мере стимулирует как обмен калия, так и обмен бромида.

ОПЫТЫ ПО ОПРЕДЕЛЕНИЮ ДИАПАУЗЫ В ВЕНГЕРСКОЙ ПОПУЛЯЦИИ GRAPHOLITHA DELINEANA WALK. (= SINANA FELD.), LEPID.: TORTRICIDAE)

ДЬ. ШАРИНГЕР, Б. НАДЬ

Диапауза *G. delineana* Walk. (= *sinana* Feld.), начинающаяся в конце августа, определяется в первую очередь фотопериодом, имеющимся во время развития личинки. Критическое с точки зрения диапаузы время освещения продолжается около 14—15 часов. При повышении температуры, влияющей на вызванную фотопериодом диапаузу, снижается эффект фотопериода. *G. delineana* является длиннодневным насекомым, имеющим факультативную диапаузу. 1—10% личинок даже в длиннодневных условиях диапаузируют, в то же время в короткодневных условиях тоже обнаружили свежую куколку на открытом воздухе (15-ого сентября). Диапаузирующие личинки без влияния холода после 1—3 месячного состояния покоя могут развиваться в имагинальную стадию. В южновенгерских районах, где возделывают коноплю, с точки зрения диапаузы личинок период с 20-ого августа до 7-ого сентября является критическим. В этот период снижается эффективная длина дня с 15 часов до 14 часов. Более ранняя уборка конопли на волокно (в первой половине августа) предотвращает перезимовку большого числа личинок. Таким образом, численность популяции следующего года может быть значительно понижена.

ИЗМЕНЕНИЕ АКТИВНОСТИ ОКСИДАЗЫ И ПЕРОКСИДАЗЫ ГЛИКОЛЬНОЙ КИСЛОТЫ В ЛИСТЬЯХ КУКУРУЗЫ ВО ВРЕМЯ ВЕГЕТАЦИОННОГО ПЕРИОДА

Л. ДЕЖИ

Изучалось изменение активности оксидазы и пероксидазы гликольной кислоты, а также содержание хлорофилла у основания и в верхушечной части листьев кукурузы в течение вегетационного периода. Установлено, что активность оксидазы гликольной кислоты всё время была выше у основания листа, то есть в молодой части, близкой к стеблю, чем в более дальных, верхушечных тканях. Однако содержание хлорофилла в верхушечных, более старых тканях было выше. На каждом отдельном уровне листьев параллельно со старением листьев снижалась активность оксидазы гликольной кислоты, в то же время содержание хлорофилла по существу не изменилось. В начале вегетации в листьях, появляющихся во время образования стебля, активность оксидазы гликольной кислоты была гораздо выше, чем в более позднем периоде, в листьях, образующихся после цветения, опыления. На основании этих данных можно установить, что во время развития кукурузы в листьях не имеется тесной связи между активностью оксидазы гликольной кислоты и содержанием хлорофилла. Далее можно установить, что степень активности оксидазы гликольной кислоты определена не возрастом листьев, а физиологическим возрастом растений. Во время развития кукурузы в листьях активность оксидазы гликольной кислоты меняясь подобно содержанию К, в противовес содержанию Са и NO_3-N. На основании этого можно предположить некоторую связь между активностью оксидазы гликольной кислоты и содержанием питательных веществ. В ходе вегетации между возростом листьев и величиной активности пероксидазы не всегда найдена тесная зависимость, параллелизм. Также внутри одного листа кукурузы не всегда более старная часть ткани обладает повышенной активностью пероксидазы. Дальнейшая задача — подробнее изучить уровни пероксидазы и открыть причины изменений активности в период развития кукурузы.

ЭФФЕКТ ГУАНИДИНО-МЕТИЛИРОВАННЫХ АРГИНИНОВ НА РОСТ ТКАНЕВОЙ КУЛЬТУРЫ ТАБАКА

Е. ТИХАК, М. МАРОТИ, Д. ВАГУЙФАЛВИ, Ш. БАЮС, А. ПАТТИ

При изучении эффекта Л-аргинина и гуанидино-метилированных производных на культурную ткань табака авторы установили, что ММА (**N_G-monometil-L-arginin**) и ДМА' (**N_G, N'^{G}-dimetil-L-arginin**) в концентрации 10 и 100 ппм в 41 и 62-дневной культуре вызвал торможение роста. Удалось выявить как в культуре ткани, так и в спиртовом экстракте питательной среды Л-аргинин и два гуанидино-метилированных аргинина методом послойной хроматографии. Можно предположить, что гуанидино-метилированные аргинины вследствие плохого деметилирования влияют на антагонизм лизин-аргинина, таким образом они представляют собой компонент системы, регулирующей естественный рост.

ИЗУЧЕНИЕ ЭФФЕКТА МЕСТА ПРОИЗРАСТАНИЯ У SOLANUM DULCAMARA

И. МАТЕ МЛ., ДЬ. ТОТ, Ш. ВАЙДА, И. МАТЕ

Авторы в этой статье изучали продуктивность растительных популяций, созданных вегетативным размножением из томатиденольного главно-агликонного таксона **Solanum dulcamara L.**, который содержит и соласодин и соладулцидин, в зависимости от места произрастания. Установили, что как на вес растения, так и на продукцию алкалоида в очень большой степени влияют условия среды. Авторы пытаются выразить экологическое влияние на продукцию (в первую очередь осадками и почвой) соответствующими для сравнения цифровыми данными.

КАКУЮ РОЛЬ МОЖЕТ ИГРАТЬ ГИББЕРЕЛИН В ПРЕКРАЩЕНИИ ПЕРИОДА ПОКОЯ КАРТОФЕЛЯ?

I. Физиологический эффект GA_3 на углеводный обмен, активность амилазы и мыхания в прорастающем картофеле

И. САЛАИ, М. НАДЬ, М. ГЕЛЬФРИХ

Авторами изучались возможная роль GA_3 в углеводном обмене, активность амилазы и повышение дыхания. Было обнаружено, что GA_3-обработка вокруг почек и их близости повышала дыхание в запасных тканях по сравнению с контрольными клубнями. Параллельно с повышением интенсивности дыхания активность амилазы, гидролизирующей крахмал, тоже увеличивается, и наиболее сильно это было выражено вокруг почек и в меньшей степени в запасных тканях, раположенных далее от них. Все эти эффекты не наблюдались, если одновременно с GA_3-обработкой использовали АВА, которое полностью ингибировало эффект, произведенный GA_3. Количество редуцирующего сахара зависело только в незначительной степени от увеличения pH, вызванного GA_3-обработкой, и в большей степени — от концентрации GA_3. Эксперименты, проведенные со срезами ткани клубней картофеля в стерильных условиях, показали, что эффект GA_3 появляется при повышении «de novo» синтеза молекул амилазы. Синтез амилазы, индуцирующей эффект GA_3, может быть компенсирован АВА.

ОПРЕДЕЛЕНИЕ ПОКАЗАТЕЛЕЙ НОРМЫ ПОВЕДЕНИЯ КРУПНОГО РОГАТОГО СКОТА РАЗНОГО ВОЗРАСТА И ИСПОЛЬЗОВАНИЯ

Й. ЦАКО

Адаптационная способность крупного рогатого скота разного возраста и использования к примененной технологии может быть определена, в первую очередь, при помощи показателей поведения, то есть можно решить до какой степени удалось создать гармонию технологических систем и физиологических потребностей животных. Изучались главные показатели повдения (лежание, движение, употребление пищи и воды, жвачка, дефекация и мочеиспускание) разновозрастных телят и телок, коров, воспитанных разным способом, и также бычков с разным живым весом. Данные являются характерными для изученных групп животных, т. к. на основании статистической оценки число наблюдений было достаточным для обобщения. Из данных видно, что разный способ воспитания не модифицирует главные процессы жизни, если потребность животных удовлетворена. Для венгерской пестрой породы в условиях Венгрии показатели воспитания телят, содержания и откармливания коров являются характерными. В отношении длительности и частоты лежания и движения, числа, дефекаций и мочеиспусканий разные генотипы ведут себя приблизительно одинаково. Таким образом, для технологического планирования, данные, полученные по венгерской пестрой породе, можно распространить и на другие генотипы.

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GYULA MÉSZÖLY

1910—1974

Dr. Gyula Mészöly, academician, titular professor, the founder, scientific adviser and director during 30 years of our Institute, originator of the Hungarian horticultural plant breeding school, deceased on March 17, 1974 after long sufferings.

A scholarly life rich in struggles but also full of success, filled with deep understanding of mankind ended on this day.

Gy. Mészöly was born at Vöröspuszta (Suhopolje) January 6, 1910. His life was not easy. Being orphaned early he was brought up in a war-orphans' home at Ikervár where he first got acquainted with gardening to devote later a whole lifetime to it.

After finishing a gardener's training school at Baja he received his diploma in the School for Horticulture, the predecessor of the present University of Horticulture, in 1935.

He worked first in a farm of Derekegyháza belonging to Ödön Mauthner Seed Co. Ltd., and later in an orchard at Érd.

In 1936 he was appointed teacher to a Horticultural Secondary School at Baja. It was during this time that he realized for the first time that advance in vegetable growing can only be achieved by introducing new varieties into cultivation.

In 1940 he came to Kecskemét and taught in the Agricultural Teachers' College. At this time he had begun his comparison trials of vegetable varieties. Results prompted him to try to increase the productivity of varieties by breeding.

He had every possibility open before him as he was charged to organize an Experimental Farm at Kecskemét in 1943.

The Second World War destroyed the first achievements. Work was taken up, however, with undiminished energy on fields devastated by shells.

His whole life exemplifies how faith, will-power and competence prevail over difficulties.

It was with hard work and an enthusiasm born of a sense of vocation that he participated in postwar reconstruction. His desire to work coupled with love for mankind and the wish to help, gave him strength to fulfil his tasks.

He respected the simple farmers who fought day and night against drought and sand blast for their livelihood and he did his best to improve their condition of life.

He called attention to the advantages of good varieties, improved seeds and up-to-date cultural methods to increase yield.

His door was always open to those who wanted to learn. He began his career by testing 900 vegetable species and varieties respectively, collected in the region; in 1947 he already directed national-wide tomato growing trials.

In 1951 two of his tomato varieties — "Kecskeméti 363" and "Kecskeméti 364" — received state registration. The variety "Kecskeméti 363" had a considerable part in improving the processing base material in Hungary and Bulgaria alike.

In 1950 the Experimental Farm was enlarged and in 1955 it was replaced by the Agricultural Experimental Research Institute of the Danube—Tisza Midregion. In the new establishment besides vegetables fruit trees and field plants had also a part. Under the direction of Gy. Mészöly the Institute soon became known all over Europe.

He was the first to develop species-hybrids with *Lycopersicum peruvianum* in Europe. Later by combining *L. hirsutum* species-hybrids "complex" species-hybrids were developed which, even today, are important sources of TMV, *Cladosporium* and *Alternaria* resistance.

He especially stressed the importance of improving quality factors and developing new types suitable for machine harvest. He established a tomato seed production unit, which can supply the whole country with first propagation seed.

As a researcher he was unselfish and ready to help. His whole being inspired joy of work and love of profession. With his colleagues he developed 14 state registered varieties and 12 variety candidates. He published 50 scientific articles, 40 popularizing articles about cultural methods and was co-author of 7 books. Besides his breeding work he had several duties elsewhere as:

— chairman of the Agronomical Division of the Hungarian Academy of Sciences

— chairman and member of the Plant Breeding Committee of the Agronomical Division in the Hungarian Academy of Sciences

- — member of the Horticultural Committee of the Agronomical Division in the Hungarian Academy of Sciences
- — chairman of the Bács-Kiskun County Organization of the Hungarian Agronomical Association
- — member of the Horticultural Committee of the Council for Agricultural Variety Qualification
- — head of the tomato work-group in the Horticultural Coordinating Committee
- — member of the Scientific Council of the Ministry of Agriculture and Food
- — member of the Ministerial Advisory Council in the Ministry of Agriculture and Food.

In 1953 he got his candidate's degree and in 1964 the Academic doctorate. Since 1967 he had been titular professor at the University of Horticulture. In 1968 he gave his inaugural lecture at the Hungarian Academy of Sciences. His merits in horticulture and society were recognized by

- — Medal of Labour, 1954 and 1958
- — Kossuth-prize, second class, 1956
- — Entz Ferenc Medal, 1963
- — Fleischmann Rudolf Medal, 1969
- — Tessedik Sámuel Commemorative Plaque and Mathias János Commemorative Plaque, 1972
- — Medal of Labour, gold, November 22, 1973

In 1973 at the annual meeting of the Hungarian Academy of Sciences he was elected regular member and the Council of the Horticultural University conferred an honorary doctorate on him in appreciation of his works of 3 decades.

His public activity was not limited to his plant breeding work: he had been member of Parliament for 20 years. His name and activity is inseparably connected with the recent history of Bács-Kiskun County.

He represented this County in Parliament for more than 20 years and was perpetual member of its Agricultural Committee. Due to his endeavours the villages of very poor natural sources belonging to his electoral district markedly improved.

In spite of the stress of work he remained a research worker and always found time to listen to the problems of his younger colleagues. Tomato breeding was his aim and joy in life. It was among his favourite plants that he knew relaxation. He worked with exemplary preciseness and conscientiousness. The rising sun invariably found him among his tomatoes. There he found strength to the day's work and rest after a long wearisome day.

Untiring activity, unbroken faith, enthusiasm and working ability characterized his whole life. He trained, instructed, helped. He had confidence in mankind, in his colleagues. He was full of plans for the future even when his health began to fail him. To his very last days he encouraged research work. Hungarian plant breeding has lost in him its pioneer and leader. An eminent character of tomato breeding has disappeared, but his memory, his work, his kind smile, his humanity will always be remembered.

We well knew that he was very ill, but hoped in his strength and will-power to recover. His work and memory oblige us to continue on the way shown by him to realize the aims for which he lived, worked and fought. His remembrance will always be kept.

B. Báldy

GYULA MÉSZÖLY'S LITERARY ACTIVITY

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COMPARATIVE STUDY ON THE BREEDING PERFORMANCE OF VARIOUS BREED SOWS KEPT UNDER LARGE-SCALE CONDITIONS

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In a pig-farm built for closed pen keeping, in the period between March 1969 and November 1971 the breeds of Hungarian Yorkshire pig, Swedish Yorkshire, Dutch Landrace, English Landrace, Belgian Pietrain and American Hampshire were compared for breeding performance (number of piglets born alive, their individual weight, as well as the number and weight of piglets at the age of 28 and 85 days), during pure-bred and crossed farrowings. The results of the investigations have revealed, on the one hand, that in the closed system of keeping the different sensitiveness of breeds must be reckoned with, and on the other hand, that in pig-farms of this type the crossing of breeds is recommended with the view of decreasing the losses of raising, and increasing the growth rate of piglets.

Introduction

During the past five years a totally new form of pork production has developed in Hungary. The essence of the change is that in the recently designed and built pig-farms both the breeding and fattening pigs are kept in a closed system, where — in addition — the various work processes (feeding, manure removal, ventillation) are carried out at a high level of mechanization compared with the old pig-farms.

A further characteristic of this large-scale system of keeping is the more rational organization of pork production realized in the close unity of gestation, piglet rearing and fattening. In Hungary about 300 large-scale pig-farms have been designed — and nearly all built — so far with the requirements of the above system of keeping kept in view; in most places the stocking of these pig-farms according to the planned rotation has meant a serious task from the beginning.

Initially only the quantitative aspects of stocking were considered, namely, the problem of how a sufficient number of young sows could be ensured; and in the absence of the necessary experiences and investigations, the question of suitability for closed pen keeping of various breeds reared in Hungary was almost completely left out of consideration. Meanwhile, in a number of new pig-farms rather unfavourable experiences have been obtained concerning this form of keeping breeding pigs, which can be briefly summarized in the following:

— in the sexual life of sows disorders frequently occur (intermittence of rutting, quiet rutting),

— unsatisfactory conception (frequent stoppage of heat),

— due to unfavourable conditions of conception and pregnancy the number of sows farrowing but a few piglets increases whereby the average number of piglets decreases,

— lactification often decreases in sows, and for different reasons the death-rate of piglets increases.

These unfavourable phenomena, which — after all — seriously affect the profitability of the new pig-farms established at heavy costs, induced us to compare the breeding performance of the most important Hungarian breeds with some recently imported foreign breeds.

For this work excellent possibilities were offered by the meat — type hybrid pig breeding activity of the Herceghalom Experiment Farm carried on under the guidance of the Research Institute for Animal Husbandry.

Material and Method

The Bábolna-system pig-farm functioning with 720 sows since the end of 1968 in the Herceghalom Experiment Farm serves exclusively the purposes of the above-mentioned hybrid pig production. In this pig-farm the following breeds are kept under perfectly identical conditions: Hungarian Yorkshire, Swedish Yorkshire, Dutch Landrace, English Landrace, Belgian Pietrain and American Hampshire pigs. Of these breeds the Dutch Landrace, Pietrain and Hampshire pigs were directly imported.

The Bábolna-system pig-farm is characterized by a completely closed, windowless keeping method. Each sow is kept during the period of heat and conceiving as well as in the course of pregnancy in a separate narrow pen, and also fed separately from a fixed trough.

Sows transferred to the farrowing pen are placed between protecting rails until weaning.

Piglets weaned when 28 days old are after-reared in the farrowing pen to the age of 85 days, where, however, the pens are connected with a manure passage furnished with a slatted floor.

During the whole time spent in the farrowing pen the piglets are allowed to consume granulated pig starter from the automatic feeders ad libitum.

During the investigation the following data were registered: number and individual weight of piglets born alive, further, number and weight of piglets when 28 and 85 days old.

The investigation consisted of the comparison of pure-bred with cross-bred farrowing in the above listed breeds. The effects of different factors (year, season, pen, number of farrowing) felt during the relatively long period of data collecting were eliminated by the aid of the Least Squares analysis, so the average values found in the Tables are no longer distorted by these effects. Nevertheless, in the case of imported breeds a possible negative effect of acclimatization may have remained.

The number of boars used for mating in the given period is presented in Table 1 separately for each breed.

Results

a) Pure-bred farrowing. Of the 2032 farrowings 884 were pure-bred and 1148 cross-bred. The data of the pure-bred farrowings are summed up in Table 2.

Table 1

Number of boars per breed used for breeding during the period of the investigation

Breed	Number
Hungarian Yorkshire	21
Swedish Yorkshire	13
Dutch Landrace	25
English Landrace	16
Pietrain	19
Hampshire	18

The data of the table reveal that in the described closed system of keeping the highest number of piglets — 9.58 on an average — were farrowed by Hungarian Yorkshire sows. The farrows of the Swedish Yorkshire and English Landrace breeds were 0.41—0.50 piglets less (4.3—6.0 per cent). They were followed by the Pietrain sows with an average of 8.56 piglets which were as much as 1.02 piglets behind the Hungarian Yorkshire sows (10.7 per cent).

The American Hampshire and Dutch Landrace sows were the last in order; they only farrowed 8.24 and 8.21 piglets, respectively, that is 1.34—1.37 piglets (14.0—14.4 per cent) less than the Hungarian Yorkshire sows.

Table 2

Pure-bred farrowing and piglet raising results of various breed sows

Breed of sow	Breed of boar	Number of farrowing	Number of piglets per litter	Individual weight of piglets kg	Number of piglets per litter	Individual weight of piglets kg	Number of litters	Number of piglets per litter	Individual weight of piglets kg	Loss of raising %
			when born		at the age of 28 days		at the age of 85 days			
Hungarian Yorkshire	Hungarian Yorkshire	507	9.58	1.42	8.19	6.86	453	7.32	28.15	22.60
Swedish Yorkshire	Swedish Yorkshire	144	9.17	1.46	7.73	7.06	130	7.39	29.00	21.78
Dutch Landrace	Dutch Landrace	93	8.21	1.48	6.84	7.09	82	6.18	31.13	24.00
English Landrace	English Landrace	55	9.08	1.51	8.62	7.13	54	7.03	30.65	24.30
Pietrain	Pietrain	37	8.56	1.41	7.22	7.58	31	7.13	26.51	22.03
Hampshire	Hampshire	48	8.24	1.42	6.15	6.86	44	5.16	26.43	36.07

The birth weight of the different breed piglets ranged between 1.41 and 1.51 kg.

As mentioned before, the piglets were weaned when 28 days old; at that time the farrow of the English Landrace breed was the largest with 8.62 piglets. As regards the number of piglets raised the Hungarian Yorkshire sows were in the second place with 8.19 piglets, followed by the Swedish Yorkshire sows with 7.73 and the Pietrain sows with 7.22 piglets.

In the Dutch Landrace litters only 6.84 and in the Hampshire farrows 6.15 piglets were left by the 28th day.

Loss until the age of 28 days ranged between 5.1 and 16.7 per cent. The lowest rate of loss was found in the English Landrace litters and the highest in the Dutch Landrace litters. In the Hungarian Yorkshire breed the raising loss of piglets was 14.5 per cent.

Differences in growth rate between the breeds were manifest in an interesting way in the weights of piglets at the age of 28 days. By that time the highest weight — 7.58 kg — was attained by Pietrain piglets. They were followed by the English Landrace, Dutch Landrace and Swedish Yorkshire piglets with 0.45—0.52 kg (6.0—6.9 per cent) lower weights. The lowest weight at the age of 28 days — 6.86 kg — was shown by the Hungarian Yorkshire and Hampshire piglets, which was 0.72 kg (9.5 per cent) less than the weight of the Pietrain piglets.

At the end of 57 days of post-raising in the farrowing pen the largest number of piglets was found in the Swedish Yorkshire and Hungarian Yorkshire breeds (7.39 and 7.32 respectively). They were followed by the Pietrain and English Landrace breeds with 7.13 and 7.03 piglets, respectively, then by the Dutch Landrace breed with 6.18 and Hampshire breed with 5.16 piglets.

These data show that the differences in fertility between the breeds could be seen at the age of 85 days as well — though in a decreasing measure.

The raising loss practically did not change from the first to the 85th day in the Hungarian Yorkshire, Swedish Yorkshire and Pietrain litters (21.78—22,60 per cent), while in the Dutch and English Landrace breed increased from 24.0 to 24.3 per cent. A remarkably high loss — 36.07 per cent — occurred, on the other hand, amongst the Hampshire piglets.

b) Cross-bred farrowing. Table 3 presents data on the cross-bred farrowings of sows of different breeds (1148 piglets). As seen from the table cross-bred piglets were obtained from sows belonging to five different breeds.

The Hungarian Yorkshire sows were mated with boars from all five breeds included in the experiment. As a response to this the number of piglets conceived from Dutch Landrace and Pietrain boars increased by 0.19—0.37 (1.9—3.8 per cent), while that of piglets originating from Swedish Yorkshire, English Landrace and Hampshire boars did not practically change.

After 28 days of sucking the number of piglets was generally similar to that in the pure-bred litters, only the piglets of the Pietrain and Dutch Landrace cross-breed remained 0.29—0.34 more. However, the weights of piglets at that age in all cases of crossing surpassed the weight of the pure-bred Hungarian Yorkshire pigs. The highest difference was 0.39 kg (5.6 per cent) in favour of the English Landrace crossing.

By the end of the 85 days after-raising more piglets were left in the litters than in the pure-bred litters in all cases except when cross-breeding was performed with Swedish Yorkshire boars. The greatest difference — 0.63 piglets (8.6 per cent) — was found in the case of cross-breeding with Pietrain boars, while cross-breeding performed with English and Dutch Landrace, as well as with Hampshire boars resulted in a 0.25—0.42 (3.4—5.7 per cent) piglet surplus.

At the age of 85 days the weights of piglets born from crossing exceeded in every case the weights of the pure-bred Hungarian Yorkshire piglets; in the case of the Hampshire, Dutch Landrace and English Landrace crosses the difference was 2.34—3.83 kg (8.3—13.6 per cent), while in the Swedish Yorkshire and Pietrain crosses only 0.27—0.37 kg (0.9—1.3 per cent).

As a result of cross-breeding the raising loss decreased in all cases until 85 days of age. Having mated with Swedish Yorkshire, Hampshire and Pietrain boars the Dutch Landrace sows farrowed 0.64—1.37 more piglets (7.7—16.6 per cent) than in pure-bred farrowing, and only mating with English Landrace boars did not result in more piglets.

The number of Swedish Yorkshire, Pietrain and Hampshire cross-bred piglets raised by the Dutch Landrace sows until the age of 28 days was 0.67—0.79 (9.7—10.8 per cent) higher than that of the pure-bred progeny, while their English Landrace cross-bred piglets were 0.15 fewer in number (2.2 per cent) when reaching that age.

As to the weight of piglets at the age of 28 days, while the weight of Swedish Yorkshire cross-bred piglets farrowed by Dutch Landrace sows was 0.25 kg (2.9 per cent) lower than that of the pure-bred Dutch Landrace piglets, the weight of piglets originating from the other crossings exceeded it by 0.10—0.28 kg (1.4—3.9 per cent).

At the age of 85 days the number of piglets showed the earlier developed trends, that is, the Swedish Yorkshire, Hampshire and Pietrain cross-bred piglets of Dutch Landrace sows were 1.01—1.95 more in number (16.3—31.5 per cent) than the pure-bred Dutch Landrace piglets. The number of Dutch Landrace × English Landrace piglets, on the other hand, was practically the same as that of the pure-bred piglets.

The individual weight of piglets at the age of 85 days showed — with the exception of the Swedish Yorkshire — the superiority of the cross-bred piglets. Cross-breeding with Pietrain, Hampshire and English Landrace breeds resulted in a difference of 0.43—1.05 kg (1.3—3.3 per cent), while

Table 3

Cross-bred farrowing and piglet raising results of various breed sows

Breed		Number of farrowing	Number of piglets per litter	Individual weight of piglets kg	Number of piglets per litter	Individual weight of piglets kg	Number of litters	Number of piglets per litter	Individual weight of piglets kg	Loss of raising %
Sow	Boar		when born		at the age of 28 days		at the age of 85 days			
Hungarian Yorkshire	Swedish Yorkshire	173	9.45	1.42	7.93	7.00	158	7.39	28.52	21.87
Hungarian Yorkshire	Dutch Landrace	195	9.77	1.41	8.48	7.00	193	7.74	30.61	21.53
Hungarian Yorkshire	English Landrace	103	9.43	1.44	7.96	7.25	103	7.57	31.98	19.17
Hungarian Yorkshire	Pietrain	100	9.95	1.42	8.53	6.94	100	7.95	28.42	20.32
Hungarian Yorkshire	Hampshire	146	9.63	1.44	8.29	7.11	145	7.59	30.49	21.42
Dutch Landrace	Swedish Yorkshire	42	8.85	1.46	7.54	6.84	42	7.19	30.29	19.49
Dutch Landrace	English Landrace	28	8.26	1.46	6.69	7.37	28	6.21	32.18	25.31
Dutch Landrace	Pietrain	11	9.58	1.57	7.51	7.30	11	8.13	31.56	16.60
Dutch Landrace	Hampshire	21	9.10	1.48	7.58	7.19	21	7.44	31.62	19.59
English Landrace	Swedish Yorkshire	42	9.09	1.49	7.85	7.05	41	7.66	32.73	18.67
English Landrace	Dutch Landrace	44	8.62	1.55	7.24	7.15	43	6.58	31.51	26.77
English Landrace	Hungarian Yorkshire	12	8.93	1.59	7.99	6.83	10	7.79	30.15	16.08
English Landrace	Pietrain	47	9.32	1.44	7.89	7.11	45	7.60	31.75	22.26
English Landrace	Hampshire	46	9.07	1.53	7.83	7.38	46	7.29	33.42	19.60
Pietrain	English Landrace	26	9.74	1.42	8.76	7.06	26	8.38	30.74	16.54
Pietrain	Hampshire	50	8.51	1.50	7.21	7.25	50	6.76	29.73	20,39
Hampshire	Swedish Yorkshire	20	8.29	1.41	6.42	7.10	20	6.25	29.86	24.45
Hampshire	English Landrace	19	8.53	1.52	7.14	7.13	19	6.81	30.42	19.67
Hampshire	Pietrain	23	8.63	1.41	7.08	6.83	23	6.79	28.86	23.47

the weight of Swedish Yorkshire cross-bred piglets was 0.84 kg (2.7 per cent) lower than that of the Dutch Landrace piglets.

Cross-breeding greatly improved the raising percentage of the Dutch Landrace sows. The only exception was the crossing with English Landrace boars where the raising loss exceeded the piglet loss registered in pure-bred Dutch Landrace farrowing by 1.31 per cent. In the other cases the situation was 4.41—7.40 per cent more favourable.

When crossed the English Landrace sows usually did not farrow more piglets than in pure-bred farrowing, in one case (after Dutch Landrace boars) even farrowed 0.46 (5.1 per cent) less.

The English Landrace sows, when crossed with boars of various breeds weaned less piglets after 28 days of suckling than in the case of pure-bred farrowing. The difference ranged between 0.63 and 1.38 in number (7.4—16.1 per cent).

The weight of piglets from English Landrace sows crossed with Swedish Yorkshire, Dutch Landrace and Pietrain boars, respectively, was practically the same at the age of 28 days as that of the pure-bred English Landrace piglets, while piglets originating from Hungarian Yorkshire boars were 0.30 kg (4.2 per cent) lighter, and those from Hampshire boars 0.25 kg (3.5 per cent) heavier.

Of the cross-bred piglets of English Landrace sows fewer died between 28 and 85 days of age than of the pure-bred litters, so with the exception of crossing with Dutch Landrace boars the cross-bred population was 0.26—0.76 piglets (3.7—10.8 per cent) higher. Farrows of Dutch Landrace cross were 0.45 piglets (6.4 per cent) fewer.

Weights recorded at the age of 85 days were — with a single exception — higher with the cross-bred piglets than in the case of the pure-bred English Landrace piglets. The difference was 0.86—2.77 kg (2.8—9.0 per cent) in favour of cross-bred piglets originating from Dutch Landrace, Pietrain, Swedish Yorkshire and Hampshire boars. On the other hand, the weight of piglets of English Landrace × Hungarian Yorkshire was 0.5 kg (1.7 per cent) lower.

During the period of investigation — from birth to 85 days of age — of the cross-bred piglets of English Landrace sows originating from Swedish Yorkshire, Hungarian Yorkshire, Pietrain and Hampshire boars 2.04—8.22 per cent more could be raised than from the pure-bred English Landrace piglets, and it was only the piglets of English Landrace × Dutch Landrace that perished at a 2.47 higher rate.

For reasons not to be discussed here, the Pietrain sows were only crossed with English Landrace and Hampshire boars. In the former case the number of piglets born was 1.18 (13.7 per cent) more than, while in the latter case practically the same as in the pure-bred Pietrain litters. When weaning, the English Landrace cross-bred litters were invariably larger in number (by

1.54 piglets, 21.3 per cent), but their weight was 0.52 kg (6.3 per cent) lower than that of the Pietrain piglets. In Pietrain × Hampshire crosses the number of piglets when weaned was the same as in the Pietrain litters, but the average weight of the piglets was found to be 0.33 kg (4.1 per cent) lower.

At the age of 85 days the number of piglets from Pietrain sows crossed with English Landrace boars was 1.25 (17.5 per cent) higher, and from those crossed with Hampshire boars 0.37 (5.3 per cent) lower than in the case of pure-breeding. The weights of cross-bred piglets exceeded that of the Pietrain piglets by 3.55 and 4.23 kg respectively (12.6 and 15.9 per cent).

The raising loss showed in both cases of crossing more favourable trends compared to the pure-bred Pietrain piglets (being 5.49 lower in number and 1.64 in percentage).

Finally, when examining the crossed farrowings of Hampshire sows we found that they produced 0.29 and 0.39 more piglets (3.5 and 4.7 per cent, respectively) when crossed with English Landrace and Pietrain boars, while the number of piglets originating from Swedish Yorkshire boars was essentially the same as that of the pure-bred piglets.

At the age of 28 days the number of piglets from all three crossings was 0.27—0.99 higher (4.3—16.1 per cent), and their weights — with the exception of the Pietrain cross — exceeded the weight of the Hampshire piglets by 0.24—0.27 kg (3.5—3.9 per cent).

At the end of the after-raising the cross-bred progeny of the Hampshire sows was 1.09—1.65 piglets (21.1—31.9 per cent) larger and at the same time the weight of the piglets exceeded the weight of the Hampshire piglets by 2.43—399 kg (9.1—15.1 per cent).

As a response to cross-breeding, in the progeny of the Hampshire sows the raising loss decreased by 11.61—16.40 per cent.

Discussion

The results of the comparative studies clearly show that in the described industrial like system of keeping the fertility of sows and their ability to raise the piglets show different trends compared to the capacities inherent in the breeds. These differences were particularly remarkable in pure-bred farrowing which suggests that the different degree sensitiveness of breeds to the closed system of keeping must be reckoned with.

In the comparative study this fact was most characteristically proved by the Dutch and English Landrace, and Swedish Yorkshire sows whose excellent fertility was generally known. At the same time, according to the data of herd-books the Hungarian Yorkshire sows are inferior in fertility to the former breeds. Nevertheless, at the site of the investigation, where the breeding stock was kept in an extremely closed system, the Hungarian Yorkshire sows proved to be superior to the Landrace breeds both in fertility and ability to raise the piglets.

From the point of view of developing pig keeping in Hungary this fact is undoubtedly reassuring, all the more so as it is in this breed that the quantity of young sows is sufficient to satisfy the demands of the large number of newly established industrial pig-farms. This means, however, that when planning the keeping technology of the pig-farms the requirements, sensitivity of the stock to be placed there must increasingly be taken in consideration in the future. This is even more important in developing the optimum keeping conditions of the different breeds.

Of the breeds included in the study the Hampshire sows raised their progenies with extremely great losses (36.07 per cent). This could not be explained during the investigation beyond any shadow of doubt. Some role may have been played in it by the fact that the sows had been directly imported and were not sufficiently acclimatized yet. This seems all the more probable because the system of keeping the breeding stock (in pens with runways) in the United States greatly differs from that used at the site of the investigation.

Beyond what have been told so far it can be established as a fact that in pig-farms with a closed system of keeping pure breeding is not a profitable method of commodity production due to raising losses being rather considerable in all breeds. This is confirmed by the results of examinations concerning single crosses.

On the basis of 19 crossing combinations of the five breeds the fertility and piglet raising ability of sows showed the following trends:

— Compared to pure-bred farrowing fertility improved in 9 cases, did not change in 6 cases and decreased in 4 cases.

— By the end of the after-raising period (85 days of age) the number of piglets per sow was higher in 15 cases, did not change in 2 cases and was lower in 2 cases.

— At the age of 85 days the average weight was higher in 17 cases and lower in 2 cases.

— The raising loss from birth to 85 days of age decreased in 17 cases and increased in 2 cases.

These data generally show the favourable effect of single crosses on all performances fundamentally affecting the profitability of piglet raising. But within this various combinations showed substantial differences from which some interesting conclusions can be drawn:

— A general improvement as a result of crossing was remarkable especialy in those breeds (Dutch Landrace, Pietrain, Hampshire) whose pure-bred farrows were unfavourable. This suggests that in the case of these breeds even the fertility of the sows was highly adversely influenced by the system of keeping, so the beneficial effect of cross-breeding on viability was more conspicuous than in the other breeds.

— In the case of the Hungarian Yorkshire sows it was the growth rate and viability of the progeny that improved in the first place as a result of crossing. The same applies to the piglets of English Landrace sows.

— Cross-breeding had the most consequent influence on the fertility of various breed sows and on the growth rate and viability of their progenies when carried out with Pietrain boars. In this case the fertility of sows increased by 2.6—16.6 per cent, a 8.1—31.5 per cent higher proportion of cross-bred piglets reached the age of 85 days, and their average weight exceeded the weight of pure-bred piglets by 1.3—9.1 per cent. It must be noted, however, that the piglets of Hungarian Yorkshire, Dutch Landrace and English Landrace sows originating from Pietrain boars were only 1.3—3.5 per cent heavier than the pure-bred piglets of the above breeds at the age of 85 days.

— Difference in type between the breeds providing sires for crossing were in a number of cases clearly expressed in the growth rate of the progeny. E. g. the piglets of Hungarian Yorkshire × Pietrain only reached a weight of 28.42 kg by the age of 85 days, while those of Hungarian Yorkshire × Dutch Landrace and of Hungarian Yorkshire × English Landrace 30.61 and 31.98 kg, respectively. The same tendency could be found in the corresponding crosses of Dutch Landrace and Hampshire sows.

No favourable effect was found to have resulted from the reciprocal crossing of the Dutch and English Landrace breeds. This can be explained partly by the common origin, partly by the mutual utilization of improving components in the breeding work which has resulted in the rather similar gene complement of the Landrace breeds.

— During the investigation some clear manifestations of heterosis could be observed, too. The best examples of this were provided by the crosses of the English Landrace and Pietrain breeds (Table 4). In litters originating from reciprocal crossing the number of piglets both when born and at the age of 85 days exceeded the piglet numbers of pure-bred English Landrace and Pietrain litters alike. Similar trends were shown by the weight of piglets at the age of 85 days.

Table 4

Number of piglets per litter and weight of piglets

Breed		Number of piglets per litter		
			at the age of	
Sow	Boar	when born	28 days	85 days
English Landrace	English Landrace	9.08	8.62	7.03
English Landrace	Pietrain	9.32	7.89	7.60
Pietrain	English Landrace	9.74	8.76	8.38
Pietrain	Pietrain	8.56	7.32	7.13

Breed		Weight of piglets, kg		
			at the age of	
Sow	Boar	when born	28 days	85 days
English Landrace	English Landrace	1.51	7.13	30.65
English Landrace	Pietrain	1.44	7.11	31.75
Pietrain	English Landrace	1.42	7.06	30.74
Pietrain	Pietrain	1.41	7.58	26.51

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SPONTANEOUS DIPLOIDIZATION IN HAPLOID NICOTIANA SILVESTRIS SPEG. ET COMES

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Cytological examinations were performed on haploid *N. silvestris* plants obtained by another culture and on their seed progenies. The haploid plants—unlike the diploid ones—have narrow leaves and small flowers. In their somatic cells 12 chromosomes, and in meiosis beside the univalents the formation of maximum 1—2 bivalents was observed. In a few cases, however, diploid cells with 12 bivalents were also found. Of the diversified anaphase chromosome distributions only the gametes with 12 chromosomes are probably viable. The pollen fertility of haploid plants was relatively high, 16.7 per cent, suggesting a large number of unreduced pollen grains. Secondary univalent associations (6 pairs of univalents) frequently observed in the first metaphase of haploid *N. silvestris* plants suggest that the basic number of the *Nicotiana* genus is 6.

Introduction

According to the evidence of many experimental data (Gerstel—Phillips 1958) the basic chromosome number of the *Nicotiana* genus was originally $x = 6$.

However, in the *Nicotiana* species existing today the lowest known haploid chromosome numbers are $n = 9$, $n = 10$ and $n = 12$, so *Nicotiana silvestris* ($n = 12$) is, in fact, a diploid species.

In 1943 Kostoff described the androgenic haploid of *N. silvestris* obtained by crossing *N. tabacum* × *N. silvestris* with *N. silvestris*. In the somatic cells of haploid plants studied by him 12 chromosomes were found. The number of bivalents in the pollen mother cells was 1—3. The haploid plants showed a 10—12 per cent pollen fertility. In his experiments Kostoff obtained seeds from haploid *N. silvestris* plants and raised progenies from them. Plants thus raised were mostly diploid though haploid and diploid trisomic plants occurred as well.

Recently numerous haploids were obtained from various plants by anther culture (Sunderland 1970, Heszky—Paál 1972). In an earlier paper we gave account of a *N. tabacum* L. cv. "Petit Havana" ($n = 24$) haploid produced and raised by anther culture (Maliga—Szilágyi 1973). In the meiosis of the haploid *N. tabacum* the development of 0—8 bivalents was observed which can be explained by the polyploid origin of the species.

The present paper deals with the cytology of haploid *N. silvestris* and its progenies produced by anther culture, since the cytological analysis of haploids and their progenies is expected to give further information on the basic chromosome number of *Nicotiana*.

Material and Method

The haploid plants were produced in anther culture by the method of Nitsch–Nitsch (1969) (Maliga–Szilágyi 1973). During the investigations on the mitosis the root tips of experimental plants were fixed in Farmer solution (abs. alcohol, acetic acid 3 : 1), then washed in 70 per cent ethanol and stained with the usual aceto carmin technique. In the meiosis examinations in most cases a quick staining method was used: the prepared anthers were placed on a slide and smashed in a drop of carmin. We concluded on the extent of pollen fertility on the basis of the ratio of stained to unstained empty-pollens (from 3000 pollen grains), and checked the result thus obtained in a pollen germination experiment (in a hanging drop of 5 per cent saccharose and 0.1 per cent boric acid solution at room temperature).

The microphotos were taken of fresh preparations using an MF Zeiss apparatus set up on a Zeiss Nfpk2 research microscope.

Results

1. Cytological investigation of haploid N. silvestris. Seven haploid *N. silvestris* plants were raised up to the stage of flowering. The plants were morphologically uniform, had narrow leaves and small flowers unlike diploids. In each of the 121 examined somatic cells of haploid *N. silvestris* 12 chromosomes were found (Fig. 1).

The meiosis of monoploids is usually irregular, since only one genom is present in them. For lack of homologous chromosomes univalents develop (Figs 2, 3).

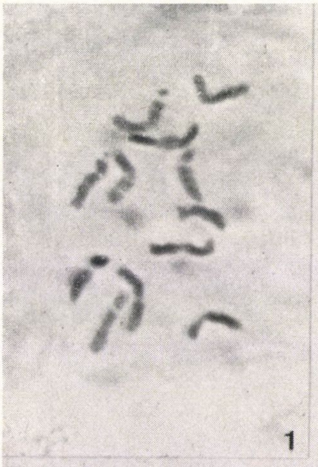
In some cases bivalents may also occur (Table 1, Fig. 4), suggesting the existence of homologous chromosome segments. The frequency of bivalents is low; of the 56 pollen mother cells examined in three cells one, in two cells two bivalents were found; there were, in addition, three diploid cells with 12 bivalents each among them.

Table 1

Number of bivalents in the first metaphase of haploid N. silvestris

Number of bivalents	0	1	2	3	4	5	6	7	8	9	10	11	12	Total
Number of PMC	48	3	2										3	56
Per cent	85.6	5.3	3.5										5.3	100

In the first anaphase the most frequent distribution of chromosomes is 5+7 and 6+6 (Figs 5, 6), from these sterile gametes develop, but even a 0+12 distribution occurs at a relatively high frequency (Table 2, Fig. 7).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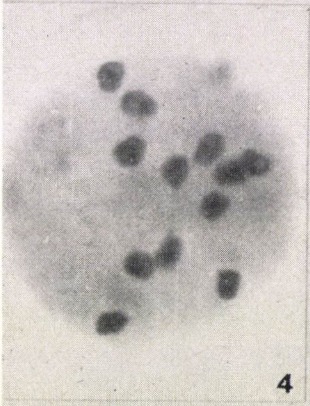
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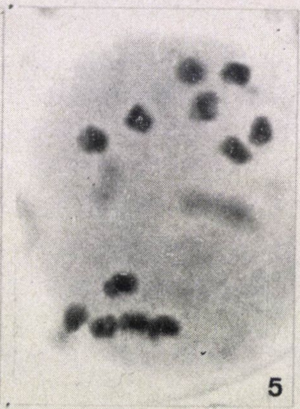
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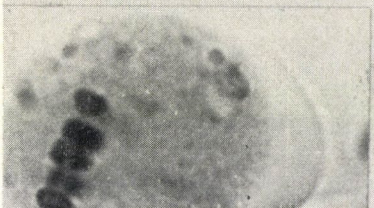
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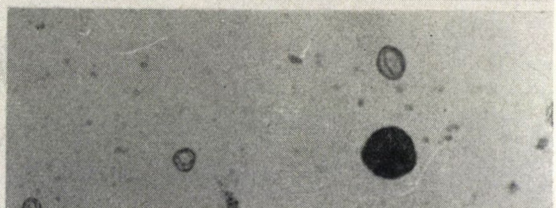
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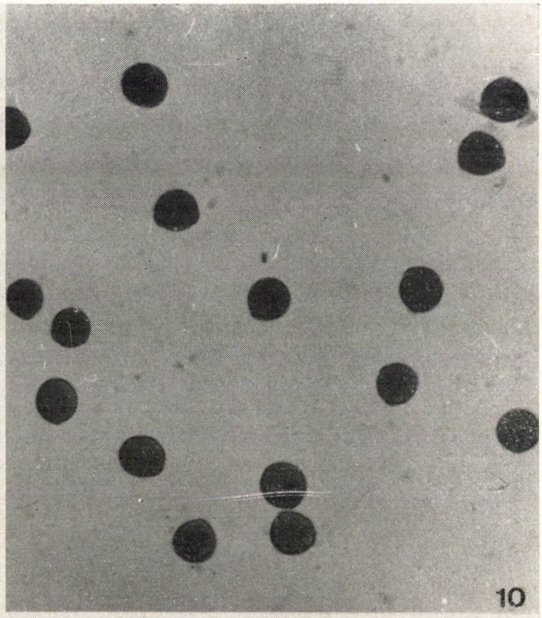
Fig. 1. Mitosis of haploid *N. silvestris* (n = 12) (× 1000)
Figs 2,3. Meiosis of haploid *N. silvestris* (diakinesis with 12 univalents) (×1000)
Fig. 4. Late metaphase with one separating bivalent (×1000)
Figs 5,6,7. The first anaphase with various chromosome distributions (×1000)
Fig. 8. Pollens of haploid *N. silvestris* (×400)



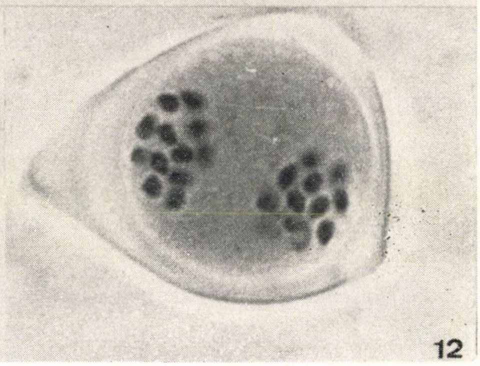


Fig. 9. Mitosis of progenies of haploid *N. silvestris* (2n = 24) (×1000)
Fig. 10. Pollens of a progeny of haploid *N. silvestris* (×400)
Fig. 11. Meiosis of a diploid progeny in the first metaphase (n = 12) (×1000)
Fig. 12. The same in the first anaphase with regular (12+12) distribution (×1000)

Table 2

Distribution of chromosomes of haploid N. silvestris in the first anaphase

Distribution of chromosomes	0 + 12	11 + 1	10 + 2	9 + 3	8 + 4	7 + 5	6 + 6	Total
Number of cells	4	4	4	11	11	23	16	73
Per cent	5.47	5.47	5.47	15.07	15.07	31.5	21.87	100

In the course of the investigation of meiosis the occurrence of normal tetrads, triads and dyads was found to be 5.7, 16.3 and 73.7 per cent, respectively. In many cases the development of micronuclei was also observed.

The haploid plants showed a 16.7 per cent pollen fertility (pointed out by staining) (Fig. 8). In the pollen germination experiment carried out in a hanging drop only the large pollen grains (supposedly containing 12 chromosomes) germinated.

From the plants marked SH_8 and SH_9 seed could be obtained after self-pollination. Generally 2500—4000 seeds develop in the capsule of the diploid *N. silvestris*. The capsules of our haploid experimental plants contained 15—20 viable seeds each.

2. *Morphological and cytological analysis of haploid N. silvestris progenies.* In our experiments the seeds obtained from the SH_8 and SH_9 plants germinated well, but the seedlings were very difficult to raise. The seedlings raised from the seeds of the haploid plant were very weak compared with the diploid seedlings, and after an occurence of *Peronospora tabacina* only five plants remained from the twenty seedlings, while of the similar age diploid seedlings not a single one died. Furthermore, their vegetation period was three weeks longer than that of the plants developed from the seeds of diploid plants. The progenies of the haploid plants were morphologically similar to the diploid control plants, but somewhat shorter.

In the somatic cells of the progenies 24 chromosomes were found (Fig. 9). Meiosis was regular: with 12 bivalents in the first metaphase (Fig. 11); in the first anaphase the distribution of the chromosomes was 12—12 in each case (Fig. 12). Pollen fertility was 99.5 per cent (Fig. 10) with full seed setting.

Thus, diploid progenies were obtained from the haploid *N. silvestris*.

Discussion

Within the *Nicotiana* genus numerous androgenic haploids have already been produced: *N. tabacum* (CLAUSEN 1924; GOODSPEED 1954), *N. glutinosa* (GOODSPEED 1954), *N. langsdorffii* (KOSTOFF 1943), *N. silvestris* (KOSTOFF 1943). According to GOODSPEED the self-pollination or crossing with diploids of the haploid *N. glutinosa* did not result in seed production; the haploid *N. tabacum* was similarly totally sterile, but in the haploid *N. silvestris* 5 per cent fertile pollen was found.

As seen from Table 1 in the meiosis of the haploid containing 12 chromosomes 1—2 bivalents only rarely occur, which suggests that even if the present basic chromosome number — 12 — were of polyploid origin (6+6), on the basis of chromosome homology hardly any trace of this is left. In the case of alloploid origin, again, more bivalents would be expected after the meiosis of diploid *Nicotiana* interspecific hybrids (SZILÁGYI 1970); at the same time secondary associations between the univalents (Fig. 3) suggest a structural likeness of chromosomes which may be related with the polyploid origin of the present basic number n = 12. This seems to be supported by the fact that the ancient basic number of *Angiospermae* was x = 7 (RAVEN—KYHOS 1965), from which an x = 6 basic number may easily be derived.

Of the chromosome distributions (Table 2) gametes with 12 chromosomes are viable and may produce progenies with 24 chromosomes which genetically will be pure homozygote diploids. In theory haploids with 6+6 = 12 chromosomes may also be produced through a generative process.

In meiosis we found dyads in a fairly high percentage. This shows again that viable gametes with 12 chromosomes may be formed, and the haploid will have diploid progenies as proved in our experiments. This was indicated by the rather high pollen fertility of haploid plants.

The meiotic course of the embryo sac was not examined, but it is probable that unreduced gametes occur here too, at least in the same proportion or even more frequently, so the production of diploid progenies with 2n (12+12 = = 24) chromosome number is possible.

Among the diploid progenies of haploid plants aneuploid plants may occur; their formation can partly be explained by the conjugation of non-homologous chromosomes and chromosome segments. At the same time it is possible that gametes with 11 and 13 chromosomes, respectively, may also produce aneuploid progenies.

This fact is known in the literature. So among the progenies of haploid *Datura* 384 diploid and 12 trisomic plants were found (BLAKESLEE *et al.*,1927) and among those of haploid *Oenothera franciscana* 711 diploids and 29 haploids (DAVIS—KULKARNI 1930).

The appearance of haploid (and aneuploid) progenies was expected in our experiments, too. This is made possible by the 6+6 chromosome distribution (with optimum chromosome composition).

It is probable that among the progenies of the haploid plant the ones that died were haploid or aneuploid plants, and only the diploids survived.

Summing up the results we can establish that in anther cultures *N. silvestris* plants with 12 chromosomes can be produced as well. The possible polyploid origin of the chromosome number 12– as shown in their meiosis– is uncertain. True bivalents hardly occur, and only the secondary associations suggest the supposed existence of genoms with 6 chromosomes. On account of its frequently occurring unreduced gametes the "haploid" *N. silvestris* (12 chromosomes) produced progenies with a chromosome number 2n = 24.

Acknowledgement

I am indebted to Dr. P. Maliga for providing the haploid *N. silvestris* and to Prof. G. Vida for his advice in my work.

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IN VITRO CULTURE OF OVARIES OF RUBUS SPECIES

By

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Ovaries excised from flower buds of the raspberry variety Malling Promise (*Rubus idaeus* L.) and of *Rubus caesius* L. before fertilization were grown on Miller and Nitsch culture media as well as on their combinations. The largest ovaries were obtained with the culture medium Nitsch + IAA 2 ppm, due mainly to the intensive callus formation occurring on this culture medium, however the size and colour of the fruits did not attain the level of those (druplets) developing under natural conditions. The fruits did not contain viable embryos, i.e. they proved to be parthenocarpic. On the Nitsch + IAA 0,1 ppm culture, however, a medium shell formation of the seeds was observed after two months. From more developed ovaries excised from larger flower buds larger fruits were formed. On a Nitsch + IAA culture medium of 0.1 ppm some R. caesius ovaries survived for a year without inoculation. This surprising phenomenon calls attention to the possibly high importance of providing sterile conditions when storing fresh fruits.

Introduction

The first successful ovary culture is connected with the name of La Rue (La Rue 1942.). The method of culturing ovaries was subsequently extended to include other species, too. The in vitro cultures were especially successful when the ovaries *(Lycopersicum esculentum, Cucumis anguria, Phaseolus vulgaris, Fragaria* sp., *Nicotiana tabacum)* were placed on to the culture medium several days after pollination (Nitsch 1949, 1951); the tomato ovaries developed into mature seedless fruits without pollination, but such fruits were smaller than those containing seeds (Nitsch 1949, 1951).

It can be said, in general, that fruits developing under *in vitro* conditions are smaller than the natural fruits — as seen e.g. in the case of strawberry (De Capite 1955), *Lycopersicum pimpinellifolium* (Jansen—Bonner 1949) and *Tropaeolum majus* (Sachar—Kanta 1958). There are, however, known cases of ovaries developing into fruits of natural size (Chopra 1958, Maheswari—Lal 1961, Johri—Sehgal 1963).

Of the fruit species grown in Hungary — apart from the above mentioned strawberry — *in vitro* cultures of ovaries of apple (Pierik 1970) and black currant (Zatykó, unpublished) are known. No data have been found, however, on the third important group of small fruits, the *Rubus* species, which form the subject of this paper.

The fruit of the raspberry is a true compound fruit consisting of small stone fruits (druplets) developing from the ovaries, therefore an *in vitro* culture of the ovaries renders the thorough study of fruit development under controlled conditions possible. It is expected, further, that on a suitable culture medium haploid plants developed from the unfertilized ovules.

Material and Method

From fully developed flower buds of the raspberry variety Malling Promise (*Rubus idaeus* L.) and of *Rubus caesius* L. ovaries were excised under sterile conditions mostly after Nitsch's method (NITSCH–NITSCH 1969). The ovaries were placed on Miller- and Nitsch-culture media (MILLER 1967, NITSCH–NITSCH 1969) as well as on the latter's variations, and kept in low light intensity (1500 lux) at temperatures of 25—27°C during the period of the experiment. To register the rate of development samples were taken of the ovaries every 2—4 weeks depending on the nature of the experiment, and 20 measurements per treatment taken of their size by means of a stereo-microscope. Samples required for the cytological examinations were taken at the same time. The ovaries fixed in Carnoy solution were inbedded in paraffine then serial sections were made of them to study the histological aspects of fruit development and the possible development of embryos. The experiments were generally evaluated after two-four weeks, but certain cultures were exceptionally maintained for months. Besides the study of the effects of various culture media the experiments presented the possibility of comparing the development of ovaries originating from flower buds of different size.

Results

On the third day after inoculation it was visible even to the naked eye that the ovaries had begun to grow, and in most cases they reached maximum size within two weeks. Some ovaries became red thus resembling the ripe fruits (druplets) of the raspberry. It must be noted that the colouring of the ovaries only occurred sporadically and even in these cases intensity did not attain the colour intensity of fruits developing under natural conditions.

Of the culture media used the ovaries showed the highest rate of growth on Nitsch + IAA (indole acetic acid) 2 ppm. (Table 1). This tendency was

Table 1

Size (mm) of Malling Promise raspberry ovaries excised from flower buds of 6—7 mm diameter when cultured on different culture media

Culture medium (ppm)	At the beginning of the experiment	At the age of two weeks
Miller	0.62	1.26
Nitsch + IAA* 0.1	0.62	1.73
Nitsch	0.62	1.70
Nitsch + IAA 2.0	0.62	2.11
Nitsch + NAA** 2.0	0.62	2.04
Nitsch + 2,4-D 2.0	0.62	1.87

* IAA = indole-acetic acid
** NAA = naphtyl-acetic acid

identical in the case of ovaries excised from flower buds of 4.5—5 mm and 6—7 mm diameter, respectively (Table 2). The latter, however, attained a larger final size — as was expected from the larger initial diameter anyway. It was not only on the size of the ovaries that the different culture media had an influence. Their effect was felt in the callus formation, too. In the first two weeks the callus formation was the most intensive in Nitsch + IAA 2 ppm. Later on a definite callus formation was observed on Nitsch + NAA 2 ppm (naphtyl-acetic acid) and even on the basic Nitsch culture medium. It was surprising, on the other hand, that on the Miller medium containing kinetin it was only after two months that any noticeable amount of callus developed. It is worth mentioning that the relatively underdeveloped ovaries — that is, those excised from smaller flower buds — displayed a much more intensive callus formation than the more developed ones. A similar phenomenon was observed in *in vitro* cultures of strawberry fruits, too (ANTOSZEWSKI—LIS 1967). On a Nitsch + IAA culture medium of 0.1 ppm the stony seedcoat developed — though mostly imperfectly — in some 50 per cent of the fruits after two months. On the other hand, in fruits of the same age, when cultured on Miller medium, not even the traces of shell formation could be found.

Table 2

In vitro growth (mm) of Malling Promise raspberry ovaries excised from flower buds of different diameter

Culture medium (ppm)	At the beginning of the experiment		At the age of two weeks	
	4.5—5 mm	6—7 mm	4.5—5 mm	6—7 mm
Nitsch	0.44	0.62	1.11	1.70
Nitsch + IAA* 2.0	0.44	0.62	1.53	2.11
Nitsch + NAA** 2.0	0.44	0.62	1.18	2.04
Nitsch + 2,4-D 2.0	0.44	0.62	1.26	1.87

* IAA = indole-acetic acid
** NAA = naphtyl-acetic acid

However, no viable embryo was found within the shell in any of the culture media, that is, the fruits developing from the ovaries were parthenocarpic. The cytological examinations confirmed that the egg cell did not even start to divide, and that later the embryo sac became degenerated (Fig. 1).

The microscopic analyses of the serial sections have confirmed our earlier supposition that the ovary of the Rubus species contains two ovules: a developed ovule and a less developed one (Fig. 2). The reason why this finding is

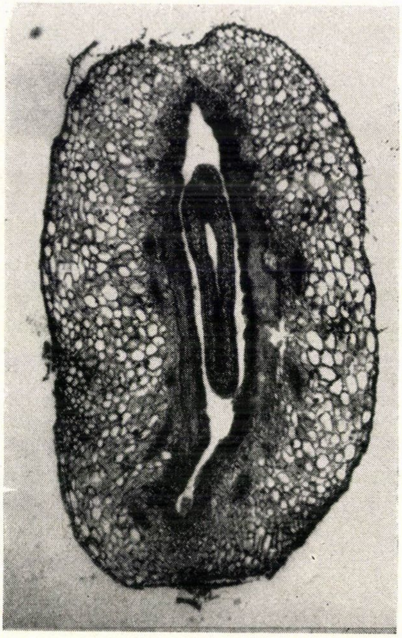

Fig. 1. Longitudinal section of a 14 days old parthenocarpic fruit (druplet) developing from a Malling Promise raspberry ovary on Nitsch + IAA culture medium of 0.1 ppm. The degenerated ovule is clearly visible in the centre. ×50

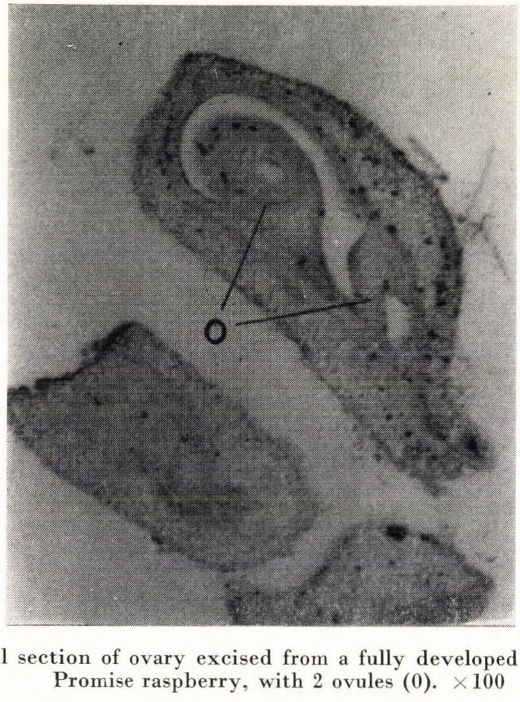

Fig. 2. Longitudinal section of ovary excised from a fully developed flower bud of Malling Promise raspberry, with 2 ovules (0). ×100

so important is that so far not more than a single seed has been found in the druplet of the Rubus species.

Some *R. caesius* ovaries were kept on Nitsch + IAA 0.1 ppm culture medium for a whole year without repeated inoculation. When evaluating the results we were surprised to find that though the growth of the ovaries had stopped, they had remained alive after such a long time. This is a remarkable phenomenon especially when we think of the fact that at room temperature raspberry fruits can only be stored for 48 hours at the most. It is possible that sterile conditions like those described above will be of importance in the future storage of fresh fruits.

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DETERMINATION OF LYSINE AND METHIONINE REQUIREMENTS

II. ESTABLISHMENT OF AMINO ACID SUPPLIES IN FATTENING PIGS BY THE DETERMINATION OF CERTAIN PARAMETERS OF THE BLOOD PLASMA

By

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The effect of amino acids applied in feed on the free amino acid of the blood plasma was studied in pigs divided into groups. In the experimental period the free amino acid composition of the plasma was determined in blood samples taken from the animals. It was found that the free amino acid content of the blood plasma was highly reactive to the different levels of amino acid applied in the diet. The optimum level of lysine (27–29 μmol/100 ml plasma) was found in the plasma when the lysine content of the feed was 0.92 per cent. The above findings agreed with the results of investigations on the nitrogen turnover. The authors' investigations suggest that the plasma can be used as a quick, precise and easy to evaluate method in establishing the amino acid requirements of pigs.

Introduction

Recently a number of publications have given account of amino acids taken up with the feed having an influence on the concentration of amino acids found in the plasma. According to MITCHELL *et al.* (1968) the more amino acids are taken up by the animal the higher the free amino acid content of the plasma becomes. The above authors as well as SZELÉNYI-GALÁNTAI *et al.* (1973) pointed out that a certain lysine content regarded as optimum occurred in the plasma in the case of maximum nitrogen retention. By measuring the isoleucine content of the plasma BRAVO *et al.* (1970) determined the isoleucine requirements of young fattening pigs. The authors studied the effect of cutting off the feed on the concentration of free amino acids in the plasma too. On the basis of experiments carried on with pigs LEUNG *et al.* (1969), and TYPPO *et al.* (1970) pointed out a correlation between the free amino acid content of the plasma and the state of amino acid supply in the animals.

Therefore, to establish the amino acid requirements of fattening pigs we studied the effect of amino acids consumed with the feed on the free amino acid concentration as well as total protein and amino acid nitrogen contents of the plasma with an experimental setting and composition of feed described in our earlier paper (SZELÉNYI-GALÁNTAI *et al.* 1973).

Material and Method

Large white meat-type pigs kept in groups were used for the blood tests. In each group five animals were marked and blood samples were taken from them before, then on 4 occasions after the experimental feed given. The blood samples were always taken 3—4 hours following the morning feeding, according to the prescription of NORDSTRÖM *et al.* (1970).

The free amino acid content of the plasma was determined with a Bio-cal 200 type automatic amino acid analyser. The free amino acids were isolated from the plasma by the modified method of MOORE—STEIN (1954; 1958): 2 ml blood plasma was deproteinized with 1 per cent picric acid and centrifugated at 6—8000 rpm. The deproteinizing substance was removed with Dovex 2×8 (120—200 mesh) resin. The free amino acids were washed off the resin with 0.06 n HCl. The solution coming off the column was evaporated (in vacuum at 60°C), then the residue taken up in 0.2 m citrate buffer of known quantity, and from this the required amount applied to the separating column.

The total protein content of the blood plasma was determined with the Biuret technique, by the method of PHILIPS *et al.* (1950), and the determination of the total amino acid nitrogen was carried out with the Folin-Danielson method after the description of BÁLINT (1962).

Results

In the experiment lysine and methionine were contained in the feed in different variations (SZELÉNYI-GALÁNTAI *et al.* 1973).

Of the results of examination concerning the free amino acid content of the blood only the changes of two amino acids, lysine and methionine, are described. The results are shown in Table 1.

Table 1

Lysine and methionine contents of pig blood plasma during the experiment (μmol/100 ml)

Blood taking	Amino acid	Group 1	Group 2	Group 3	Group 4	Group 5
Number and time		Control	Basic feed +0.3% lysine	Basic feed +0.6% lysine	Base feed +0.6% lysine +0.5% meth.	Base feed +0.5% meth.
I. 15. April	Lys.	17.2 ± 1.46	16.8 ± 1.46	16.5 ± 1.46	17.1 ± 1.46	17.3 ± 1.46
1971.	Meth.	9.5 ± 0.47	8.9 ± 0.47	10.5 ± 0.47	10.2 ± 0.47	11.3 ± 0.47
II. 1. July	Lys.	16.6 ± 1.44	25.6 ± 1.80	28.3 ± 3.05	29.8 ± 4.59	18.7 ± 0.99
1971.	Meth.	11.0 ± 1.24	10.6 ± 2.01	9.47 ± 1.51	18.6 ± 2.9	25.8 ± 5.10
III. 11. August	Lys.	17.2 ± 1.43	18.3 ± 3.03	29.7 ± 5.83	27.4 ± 0.84	18.0 ± 1.69
1971.	Meth.	6.4 ± 0.40	6.4 ± 1.71	5.60 ± 1.83	6.0 ± 0.41	10.2 ± 2.69
IV. 9. Sept.	Lys.	15.2 ± 0.80	15.5 ± 1.48	24.0 ± 2.85	20.4 ± 1.45	15.8 ± 1.49
1971.	Meth.	5.2 ± 1.03	5.4 ± 1.75	5.2 ± 1.45	6.0 ± 1.00	6.9 ± 1.49

Lys. = lysine
Meth. = methionine

The table gives the quantities of free lysine and methionine found in the plasma on the four occasions of taking blood (I, II, III and IV).

In treatment I the animals of all five groups had consumed the same composition of feed. In their blood plasma the concentration of free lysine was 16.5—17.3, while the methionine content ranged from 8.9 to 11.3 μmol/100 ml; the standard deviation (s. d.) was ±1.46 and ±0.47, respectively. The concentration of the amino acids examined in the blood plasma was nearly identical in all groups.

The lysine and methionine contents of blood plasmas taken from the control animals (group 1) did not, in essential, change during the experiment.

On the second occasion of taking blood (treatment II) the free lysine concentration of the plasma increased by an average of +8.8 μmol/100 ml in group 2, then, on the subsequent two occasions (treatments III and IV) decreased to its initial concentration.

In the case of group 3 (+0.6 per cent lysine added) the free lysine concentration of the plasma increased by +11.8 μmol/100 ml on the second (treatment II) —, and even somewhat further on the third (treatment III) occasion of taking blood, and it was only on the last occasion (treatment IV) that it decreased to 24.0 μmol/100 ml, and even then was 7.8 μmol/100 ml higher than in treatment I, that is, before the experimental feed applied.

In group 4 the lysine content in the plasmas of blood samples taken on different occasions showed a trend similar to that in group 3.

In group 5 where no lysine was supplemented, the free lysine content of the plasma did not show any substantial change during the experiment.

Any essential change in the methionine content of the plasma was only found during the experiment in the case of supplementary methionine (0.5 per cent) applied (groups 4 and 5).

The increase, however, was only apparent on the second occasion of taking blood (treatment II), while on the third and fourth occasions (treatments III and IV) the concentration of methionine in the blood plasma was much lower in all groups (groups 1, 2 and 3, too) than the initial value. In group 5 this was natural, since — having observed toxic symptoms in the animals as a response to the methionine rich feed (dysorexia, loss of weight) — we replaced the feed by that of the control animals before the fourth occasion of taking blood (treatment IV.).

Changes in the methionine and lysine concentration of the plasma during the experiment are shown in Figs 1 and 2. The total protein content of the blood plasma gradually increased in the course of the experiment, as seen in Fig. 3. The total amino acid nitrogen displayed a slight increase in the experimental period (Fig. 4).

Conclusions

In our experiments we tried to find an answer to the question how the effect of a lysine supplement (0.3—0.6 per cent, groups 2 and 3) added during the period of fattening, as well as that of methionine fed jointly with lysine

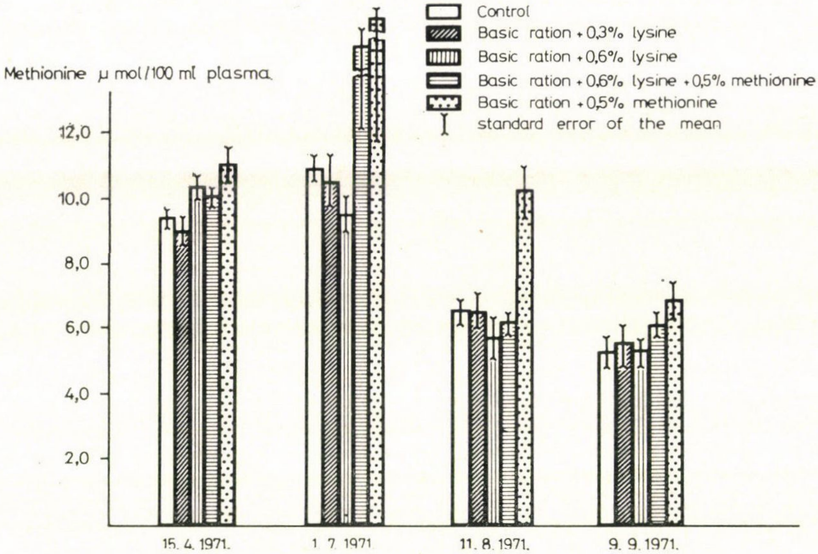

Fig. 1. Free methionine concentration in the plasma of growing pigs (µmol/100 ml)

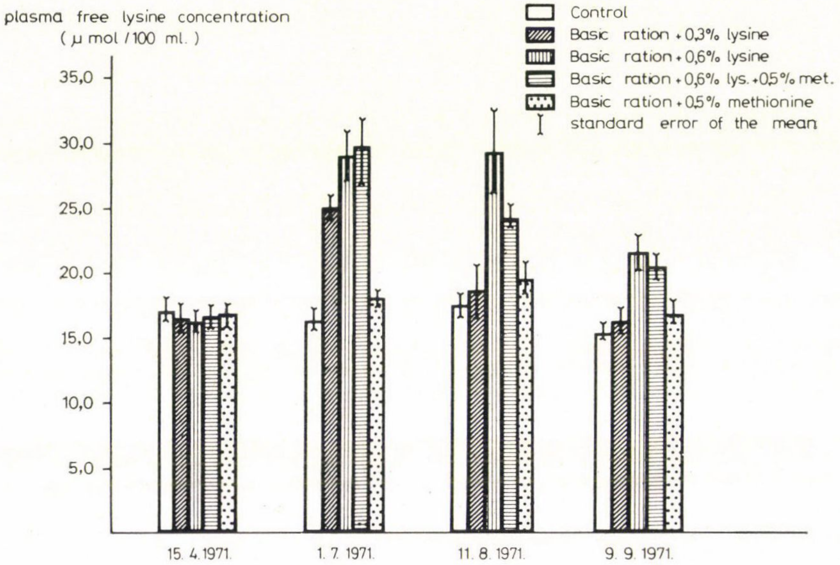

Fig. 2. Free lysine concentration in the plasma of growing pigs (µmol/100 ml)

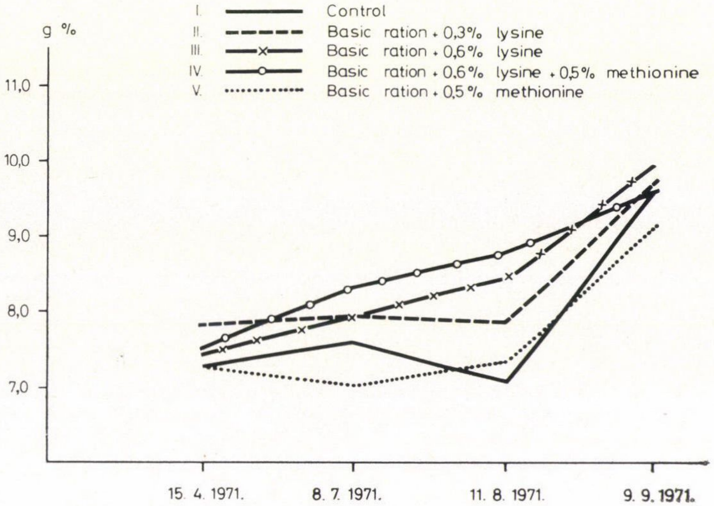

Fig. 3. Changes in the plasma protein content of growing pigs (g/100 ml)

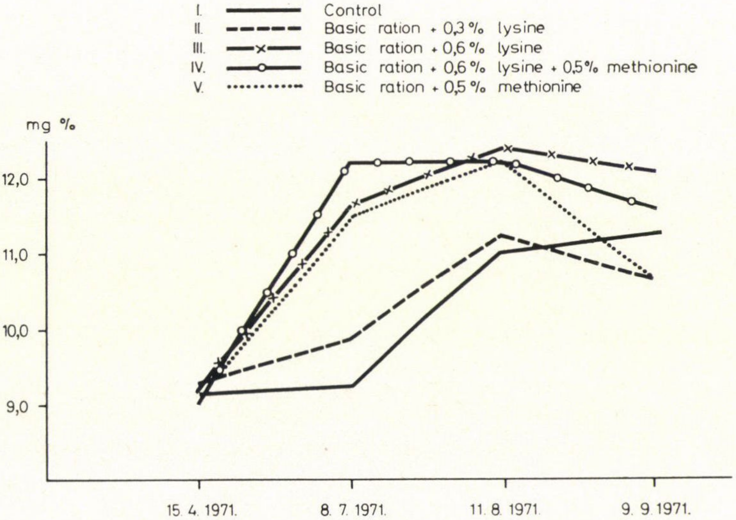

Fig. 4. Total free amino acid concentration in the plasma of growing pigs (mg/100 ml)

(0.6 per cent lysine +0.5 per cent methionine, group 4) and of a methionine supplement (0.5 per cent, group 5) would show in the plasma of blood samples taken on different occasions.

The results of the experiment showed that as a response to lysine supplementation the lysine content of the blood plasma reached a maximum then decreased in the course of fattening, but during the whole period of the experi-

Ø non significant difference
x significant $(p < 0{,}5)$
xx highly significant $(p < 0{,}1)$

I. Control
II. Basic ration + 0,3 % lysine
III. Basic ration + 0,6 % lysine
IV. Basic ration + 0,6 % lysine + 0,5 % methionine
V. Basic ration + 0,5 % methionine

	I.	II.	III.	IV.	V.
I.		Ø	Ø	Ø	Ø
II.			Ø	Ø	Ø
III.				Ø	Ø
IV.					Ø
V.					

15. 4. 1971.

	I.	II.	III.	IV.	V.
I.		xx	xx	xx	Ø
II.			Ø	Ø	x
III.				Ø	xx
IV.					xx
V.					

1. 7. 1971.

	I.	II.	III.	IV.	V.
I.		Ø	xx	xx	Ø
II.			xx	xx	Ø
III.				Ø	xx
IV.					xx
V.					

11. 8. 1971.

	I.	II.	III.	IV.	V.
I.		Ø	xx	Ø	Ø
II.			xx	Ø	Ø
III.				Ø	x
IV.					Ø
V.					

9. 9. 1971.

Fig. 5. **Effect of lysine supplementation on the free lysine concentration in the plasma of growing pigs**

Ø non significant difference
x significant
xx highly significant

I. Control
II. Basic ration + 0,3 % lysine
III. Basic ration + 0,6 % lysine
IV. Basic ration + 0,6 % lysine + 0,5 % methionine
V. Basic ration + 0,5 % methionine

	I.	II.	III.	IV.	V.
I.		Ø	Ø	Ø	Ø
II.			Ø	Ø	Ø
III.				Ø	Ø
IV.					Ø
V.					

15. 4. 1971

	I.	II.	III.	IV.	V.
I.		Ø	Ø	xx	xx
II.			Ø	xx	xx
III.				xx	xx
IV.					Ø
V.					

1. 7. 1971.

	I.	II.	III.	IV.	V.
I.		Ø	Ø	Ø	xx
II.			Ø	Ø	xx
III.				Ø	xx
IV.					xx
V.					

11. 8. 1971.

	I.	II.	III.	IV.	V.
I.		Ø	Ø	Ø	Ø
II.			Ø	Ø	Ø
III.				Ø	Ø
IV.					Ø
V.					

9. 9. 1971.

Fig. 6. **Effect of methionine supplementation on the free methionine in the plasma of growing pigs**

ment showed a higher value compared to the control. In case the feed is supplemented with a smaller quantity of lysine, the lysine level of the blood plasma reaches a maximum in an earlier phase of the fattening period, then settles at a value identical with that of the control animals.

Under the influence of a methionine supplement — when added without lysine to the animals — the blood plasma shows a high methionine level already in an early phase of fattening, which then — after a rapid decrease — reaches that of the control animals. (This can also be explained by the earlier mentioned change of feed.) Methionine, when added with lysine, showed at once in the methionine level of the blood plasma, though to a lower extent than when added by itself.

In the groups of animals not given additional methionine — just like in the control animals — the methionine content of the plasma decreased by the end of the fattening period. The plasma lysine level of groups not given lysine supplementation shows a gradual decrease during the fattening period, though this decrease is not so marked as in the case of methionine.

The results of measuring the total protein and amino acid nitrogen content of the plasma also showed that the feed given to the animals covered their amino acid requirements, but the examined parameters were not sufficient for determining the right protein and amino acid ratios.

The results of the experiments suggest that the determination of the free amino acid level in the blood plasma may be a suitable method of establishing the state of amino acid supply in pigs, as the free amino acid level of the plasma in the animals responses extremely quickly to additional amino acids. So the method may become a rapid and reliable way of determining the amino acid supplies of a stock. For the practical application of the method we have to know the amino acid composition of the feed, the breed and age of the experimental animal, and perform blood tests of at least 5 animals of the stock concerned.

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ION EXCHANGE WITH SCENEDESMUS OBTUSIUSCULUS

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In the previous experiments it was proved that the mechanism of K^+- and Br^- uptake by *Scenedesmus obtusiusculus* depends on the state of cell development. The characteristics of the concentration curves, the competition and the stimulating effect of light, indicate active processes regarding mainly the potassium. However, it is questionable whether the mechanism of bromide uptake is active or not. The results of the exchange experiments verify that the exchange of the bromide ion is more an active process than its uptake-mechanism. Light stimulates both K^+-exchange and Br^--exchange in a great measure.

Introduction

The cation and anion uptake and their uptake mechanism in unicellular green algae pose several questions. In literature different opinions can be found on the uptake activity and first of all the uptake energy.

In our institute the cation (K^+ and Na^+) and anion (Br^-) uptake of *Scenedesmus obtusiusculus* — photosynthesizing unicellular algae — is studied. During the experiments several contradicting data came up, therefore, we considered it important to check the efflux mechanism in more details, because it can be supposed that the efflux is not a simple and passive process. In this paper we shall report about the K^+ and Br^- exchange results.

Material and Method

The experiments were carried out with an algae strain — *Scenedesmus obtusiusculus* CHOD — supplied by the Biological Research Institute of the Hungarian Academy of Sciences, Tihany. The cells were grown in inorganic medium under the reported conditions (Meszes—Kralovánszky—Cseh—Böszörményi 1967). In a great majority of the experiments synchronized cells were used. The cells were synchronized by means of a method specially worked out for this purpose (Meszes—Sipos 1968, Meszes—Komárek 1970).

The cells for the exchange experiments were centrifuged (4—5000 g, 3 minutes) and washed three-times in a "Ca^{2+}—Mg^{2+}-solution". The concentration of the Ca^{2+} and Mg^{2+} ions was the same as that of the medium (Ca^{2+}:0.61 mEquiv.) liter and Mg^{2+}: 0. 41 mEquiv. (liter). After this the cells were resuspended in the "Ca^{2+}—Mg^{2+}-solution" labelled with K^{42} and Br^{82} (K^+ concentration was 0.03 mM and the Br^- conc. was 0.1 mM). The cells were incubated in this labelled solution for two hours in diffuse light, in illumination and dark. At the end of the uptake period lasting two hours definite aliquotes were filtered and washed through a membrane filter (Typ MF-50 from Membranfilter Gesellschaft Göttingen) and the quantity of the uptaken Br^{82} and K^{42} was determined in the separated cells by filtration.

After the uptake period the cells were separated by filtration and washed in "Ca^{2+} — Mg^{2+}-solution and resuspended in inactive KCl and KBr, respectively. The concentration of K^+ and Br^- was the same as that of the uptake solution. In the inactive solutions the cells were incubated in diffuse light, in illumination and in dark. The rate of exchange was studied as a function of time.

The data were calculated in unequiv. K^+ or Br^+ 10^{10} cells or the uptake and exchange of the two ions expressed in percentage as a function of time.

The K^{42} and Br^{82} ion exchange were carried out with so-called "dark and light cells" coming from the dark or light periods of the cell cycle.

Results

During the experiments with *Scenedesmus obtusiusculus* the Br^{82}-exchange was studied after an uptake period of two hours in diffuse light. After the uptake period there is a rush — a few seconds long — exchange supposedly from the free space (Fig. 1). This process takes 5 minutes, then the Br^{82} content of the cells decreases at an even rate. It is valid for both "light and dark cells".

The behaviour of the K^{42} ions during the exchange processes is interesting (Fig. 2). With the "dark cells" almost only the ions in the free space are exchanged, because there is no further exchange beginning from the tenth minute of the exchange experiment to the end of the second hour. On the other hand after a rush initial exchange the "light cells" in two hours gradually pass nearly 100 per cent of the K^{42} quantity taken up before.

As the "dark and light cells" had shown a different behaviour during the exchange experiments, the effect of the light and darkening on the exchange process was studied. As the results have shown light can increase the ion efflux in the same way as the influx.

As it is shown in Fig. 3, the Br^{82} content of the cells decreases in dark at an even rate for two hours with a rush initial exchange of the cells grown in dark at the beginning. On the other hand, the illuminated cells pass the uptaken Br^{82} in 100 per cent almost in the first 30 minutes. The "light cells" in dark only pass about 25 per cent of the uptaken Br^{82} in two hours, whereas in light they pass almost the whole Br^{82} content in 20 minutes.

The same is valid for the K^{42} exchange, too (Fig. 4). As it is shown in Fig. 4 the exchange of the "dark and light cells" in darkness hardly exceeds the quantity of ions coming from the free space. On the other hand, in light 70 per cent of the uptaken quantity is passed.

Discussion

Many papers deal with the effect of light on the ionfluxes. The dependence upon light of the ionfluxes and the inhibition of those by different inhibitors permit an insight into the relation of the ionfluxes and photosynthesis.

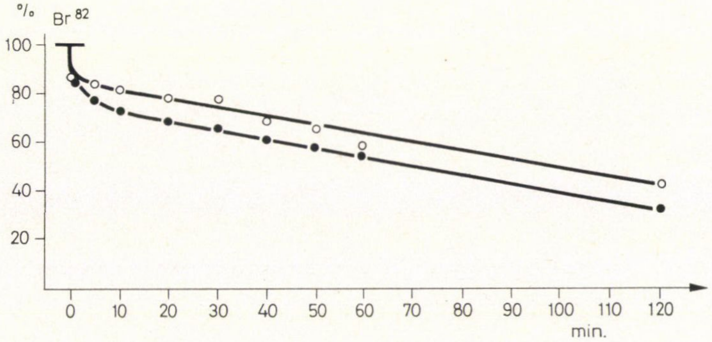

Fig. 1. The Br^{82}-exchange rate by *Scenedesmus obtusiusculus* cells, taking the Br^{82} quantity taken up in two hours for 100%. ●——● "dark cells"; ○——○ "light cells"

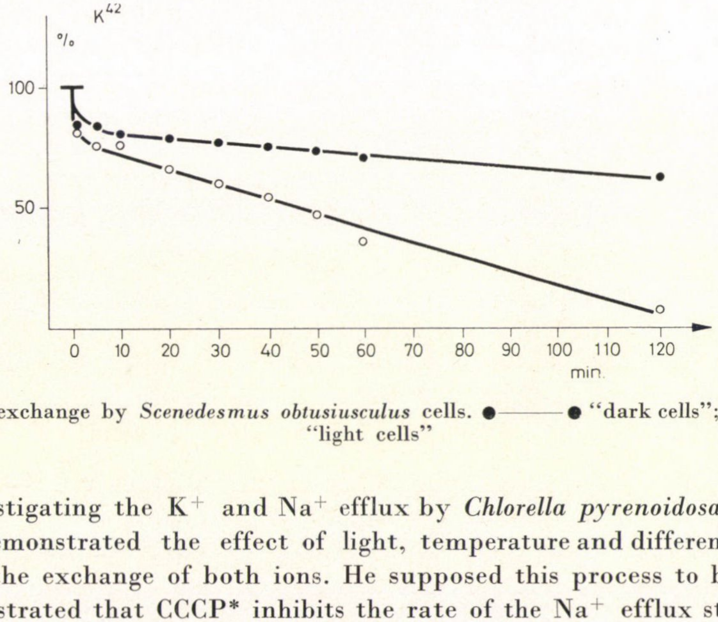

Fig. 2. K^{42}-exchange by *Scenedesmus obtusiusculus* cells. ●——● "dark cells"; ○——○ "light cells"

Investigating the K^+ and Na^+ efflux by *Chlorella pyrenoidosa* Barber (1968a) demonstrated the effect of light, temperature and different metabolites on the exchange of both ions. He supposed this process to be active. He demonstrated that CCCP* inhibits the rate of the Na^+ efflux stimulated by light to a great extent (Barber 1968b). Investigating the K^+ exchange of *Chlorella* cells stimulated by light, he supposed that the kinetics of the efflux showed that the exchange of a great part of the internal K^+ is controlled by a first-class process (Barber 1968a).

Shieh—Barber (1971) observed a net Na^+ efflux stimulation with *Chlorella* cells induced by K^+ in light. Remarkable results were reported by Rybová—Janácek—Slaviková (1972) with *Hydrodictyon reticulum* cells. It was established that the K^+, Na^+ and Cl^- flows namely their in- and efflux were light dependent and decreased by darkness. From these experiments the

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* CCCP = carbonylcyanide-m-chloro-phenylhydrazone.

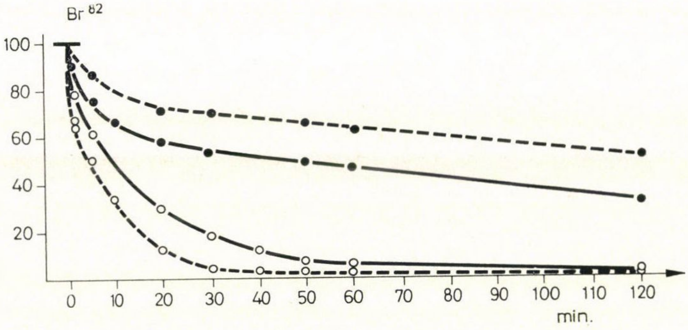

Fig. 3. The effect of lighting and darkening on the Br^{82}-exchange by *Sc. obtusiusculus* cells
●———● exchange of "dark cells" in dark; ○———○ exchange of "dark cells" in light;
●- - - - -● exchange of "light cells" in dark; ○- - - - -○ exchange of "light cells" in light

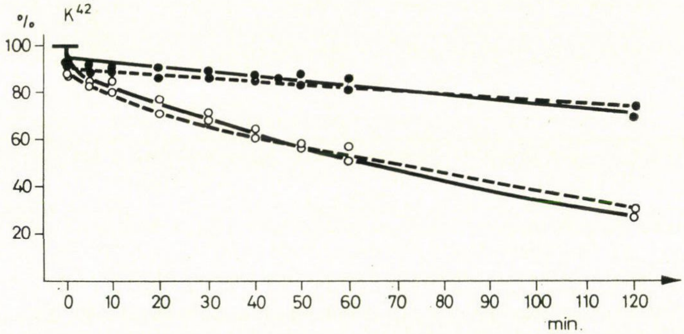

Fig. 4. The effect of lighting and darkening on the K^{42}-exchange by *Sc. obtusiusculus* cells.
●———● exchange of "dark cells" in dark; ○———○ exchange of "dark cells" in light'
●- - - - -● exchange of "light cells" in dark; ○- - - - -○ exchange of "light cells" in light

conclusion was drawn that the ion flow in both directions takes place almost exclusively through active pumps coupled metabolically.

According to our results the mechanism of the ion uptake with *Scenedesmus obtusiusculus* depends on the state of cell development. The cells grown in light and dark show a typical change in uptake in the function of growth and division of cells. In the early light period the uptake intensity is smaller, then the K^+ and Br^- uptake of cells gradually increases parallelly with the cell volume enlargement (MESZES—KRALOVÁNSZKY—CSEH—BÖSZÖRMÉNYI 1967). In the period of the formation of the daughter cells the uptake decreases again in the function of concentration. According to the concentration curves of the uptake after a saturation the Br^- uptake with *Scenedesmus obtusiusculus* increases again at a high concentration. In the investigated concentration range the K^+ and Na^+ uptake with *Scenedesmus* shows a saturation characteristic. The K^+ and Na^+ ions mutually inhibit the uptake of one another (MESZES—KRALOVÁNSZKY—CSEH—BÖSZÖRMÉNYI 1967).

In spite of the repeated experiments it remains questionable whether the uptake with the investigated ions is active or not although the cells show competition between chloride and bromide, the concentration curves are saturated, light increases the uptake and all these things indicate an active process. Mainly the bromide uptake is problematical because the linear part of the time curves cannot be demonstrated in certain cases. But we cannot explain the increases of the K^+ influx by "light cells" after a five minutes pretreatment in dark either when the ligh⇄dark transitional effect is studied (Meszes—Cseh 1973). For this reason it was important to investigate the exchange of both ions before the continuation of the uptake experiments.

As it is shown in the figures, light has the same effect on the Br^- and K^+ uptake and exchange with *Sc. obtusiusculus* cells. Light stimulates both the influx and efflux of both ions. After a rush exchange of Br^- and K^+ ions a slow pass with both "dark and light cells" can be observed for two hours, on the other hand, 100 per cent of the K^{42} and more than 70 per cent of the Br^{82} content taken up in light is passed. This stimulating effect of light refers to an active exchange process. Further these results — confirming the earlier data — show that the ion uptake mechanism with these algae depends on the state of cell development.

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DIAPAUSE EXPERIMENTS WITH GRAPHOLITA DELINEANA WALK. (= SINANA FELD., LEPID.: TORTRICIDAE) POPULATIONS IN HUNGARY

By

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The diapause of *G. delineana* Walk. (= *sinana* Feld.) beginning at the end of August is primarily determined by the photoperiod prevailing during the development of the larvae. The time of illumination critical for the diapause is between 14 and 15 hours. Temperature influences the diapause induced by the photoperiod in such a way that higher temperatures decrease the photoperiodic effect. *G. delineana* is a long-day insect which has a facultative diapause. Larvae diapaused to 1—10 per cent even on long days, at the same time fresh pupae were observed in the open on short days as well (15 September). Without a cold effect the diapausing larvae may develop into imagos after 1—3 months of rest. In the hemp producing regions of southern Hungary the period between 20 August and 7 September is critical for the diapause of the larvae. In this period the effective day length is reduced from 15 to 14 hours. An earlier harvest of fibre hemp (in the first half of August) prevents the overwintering of large masses of larvae, and the population of the following year can thereby be considerably reduced

Introduction

G. delineana Walk. (= *G. sinana* Feld., *tetragrammana* Stgr.) appeared as a new pest on hemp (*Cannabis sativa* L.) in South-East Europe in the middle of the sixties (ZAYETZ 1964, MANOLACHE *et al.* 1966, NAGY—REICHART 1966, BES 1967, NAGY 1967a, b, TZIBULSKAYA 1968, etc.). It had been known for some time on hop (*Humulus lupulus* L.) (SWATSCHEK 1958). The taxonomic and ecological aspects of populations living on the two host plants are just as much unexplained as their appearance as a pest in Europe. In China it was already known earlier as a pest of hemp seed (TSAO 1963).

The preliminary results of our investigations on the diapause of *G. delineana* were briefly reported earlier (SÁRINGER—NAGY 1971). In the present paper, after a description of the methods of investigation, a more detailed interpretation of the data and results is given.

Acknowledgement of the diapause plays a part in the correct interpretation of pest signalization too, which can be carried out with various methods (NAGY 1971).

Experiments with *G. delineana* had a double aim: on one hand, to find out what ecological factors the diapause of caterpillars, beginning from the end of August depended on; on the other hand, whether a temperature below zero was needed for the reactivation of larvae already diapausing.

Material and Method

The population serving as a starting point for the experiments originated from the district of Tótkomlós-Battonya (South-East Hungary). The imagos collected in the open laid eggs on hemp leaves on 28–29 May 1967. The eggs, together with the leaves were kept in an aqueous higrostate in the laboratory. On the day of hatching the larvae were placed in groups of 35–50 on a young hemp shoot fixed by a cotton plug into a water bottle, by means of a fine brush. The hemp shoot was placed in a 24 cm high and 12 cm diameter glass cylinder closed from below by a Petri-dish lined with filter paper, and from above by a piece of linen fixed with a rubber ring (Fig. 1). When the hemp shoot began to wilt a new shoot was placed close by the side of the wilting ones, so the larvae were able to climb by themselves to the fresh hemp. As long as the larvae were feeding every two or three days a new hemp shoot was placed in the culture. The wilted hemp was always left in the culture pot. When the larvae were fully developed, the wilting and drying plant parts, together with the larvae, were placed in a glass vessel of 13 cm height and 13 cm diameter, and covered with cellophane. In order to ensure an optimum vapour content a glass tube half filled with water and closed with a cotton plug was placed in the culture pot. 25–60 days after the last moths appeared the dry hemp shoots and leaves were examined carefully, and the number of diapausing larvae determined.

It should be noted that under such conditions the development of larvae differs from that taking place in the open, where in most cases the larvae develop in a several cm passage made in a single plant without changig their place. However, this fact is not very likely to have influenced the results of the diapause experiments.

Investigations were made with the above method in all three generations, in thermostates of 18 ± 1.7, 23 ± 1.2 and 28 ± 0.7°C temperature, respectively, with daily photoperiods of 12/12, 13/11, 14/10, 15/9, 16/8 and 17/7 hours.

Similar cultures were set up in the open (control cultures) which were exposed to natural day light and day length as well as to outdoor temperature.

Both outdoor and laboratory experiments were performed in the Laboratory of the Research Institute for Plant Protection, Keszthely (South-West Hungary). The natural day lengths at Keszthely agree with those at the site of origin of the populations included in the experiment.

By the application of the above culture method 20–80 per cent of the initial larva population developed, 27–63 per cent of the diapausing larvae wove inside the hemp stalk, while the others in the fallen hemp leaves. In one case a diapausing larva was found inside the hemp seed. About one-third of the larva and cocoon respectively, protruded from the seed.

Results

As a result of investigations made with three summer generations, at three constant temperatures and in six different photoperiods it can be established that the diapause setting in at the stage of full development in a larva is primarily determined by the photoperiod prevailing during the larval development (Fig. 2). Higher temperatures to some extent decrease the effect of the photoperiod on the diapause. This is especially obvious in the case of the first summer generation where a temperature of 28°C with a photoperiod of 12/12—14/10 hours only resulted in a 70—75 per cent diapause. The lower the temperature the higher the effect of the photoperiod on the diapause.

The time of illumination critical for the diapause is between 14 and 15 hours. Daily illumination of 14 hours and less, however, only resulted in a 100 per cent diapause at 18°C. 1—10 per cent of the larvae raised under a daily illumination of 15 hours or more remained in diapause. Thus, according to the laboratory examinations *G. delineana* is considered a long-day insect which has a facultative diapause. Diapause responses given by the insect to various

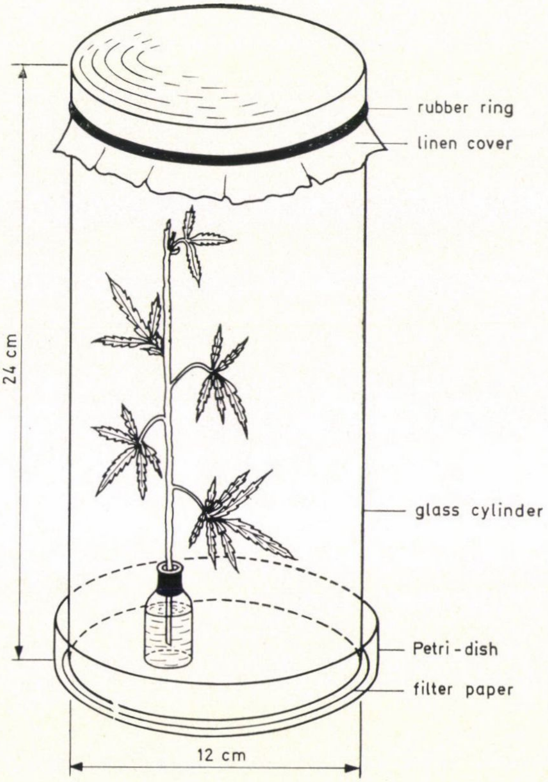

Fig. 1. Culture pot for rearing *G. delineana*

ecological conditions show that the reactions of the population are not uniform. Similar results were obtained in diapause studies performed with the species *G. funebrana* Tr. to (Sáringer 1967, 1970, 1971, Sáringer—Deseő 1968, Deseő *et al.* 1971).

Diapause percentages determined in an outdoor insectary (i. e. under changing temperature and natural photoperiodical conditions) are shown in Table 1. According to the data 94.5 per cent of the second summer generation placed out at the egg stage on 9 August displayed a diapause, while 100 per cent of the third summer generation placed out at the egg stage on 24 August. Populations set in later also showed a 100 per cent diapause.

The reactivation (further development) of larvae in diapause does not require any — longer or shorter — period of temperatures below or around zero, although the reactivation of caterpillars, which either had not been cooled at all or had been cooled for a short time then kept at higher temperatures, lasted for a longer period. At a temperature of 18°C or more diapausing larvae may develop into imagos after 1—3 months of rest. This fact makes it

Table 1

Diapause percentages of Grapholitha delineana larvae in an outdoor insectary under natural photoperiodical and temperature conditions (min.: 16.1, max.: 29.5°C, Keszthely)

Time of placing out eggs and L_1-s	Generation	Diapause %	Effective day-length
9 August	Summer II.	94.5	15^h 34′
24 August	Summer III.	100	14^h 49′
1 September	Summer III.	100	14^h 23′
11 September	Summer III.	100	13^h 49′
17 September	Summer III.	100	13^h 28′

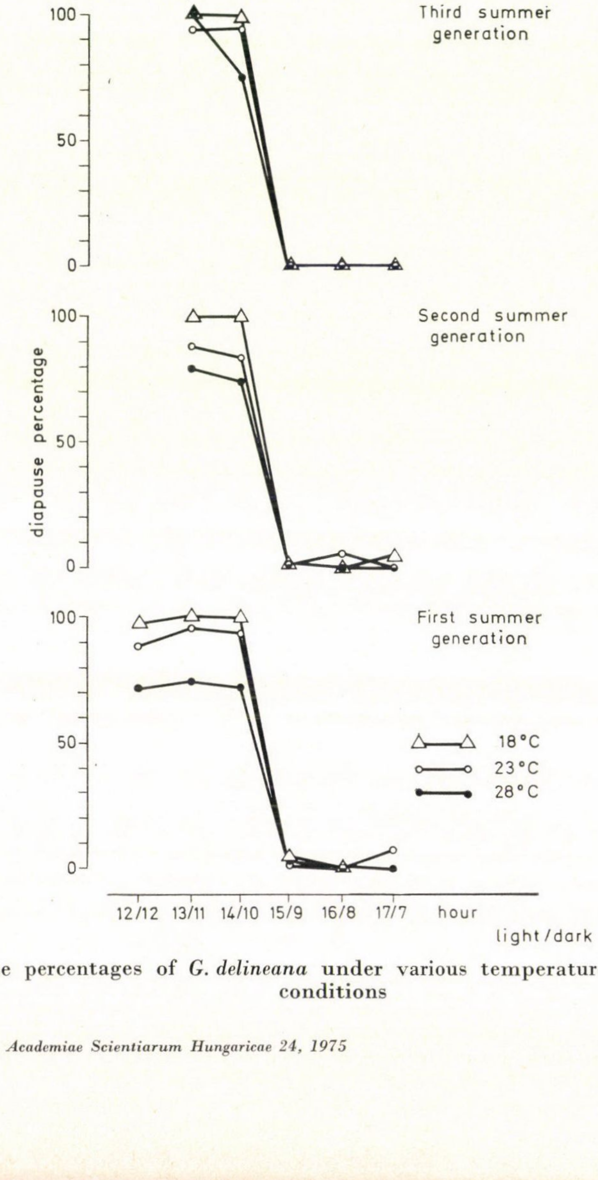

Fig. 2. Diapause percentages of *G. delineana* under various temperature and photoperiod conditions

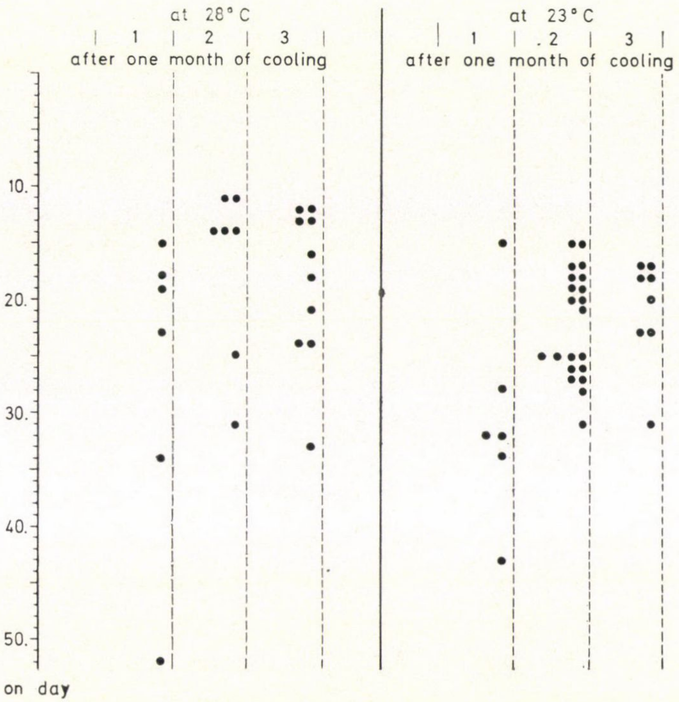

Fig. 3. Distribution in the time of appearance of *G. delineana* moths from caterpillar populations kept under identical conditions

difficult to determine to which generation the specimens caught in the open belong to.

In order to determine the length of the reactivation period more precisely an infected hemp material of the same origin, collected in the field on 15 September, was placed in thermostates of 23 and 28°C respectively (with a daily photoperiod of 17/7 hours). The experimental material had previously been stored for 1—3 months in a refrigerator at temperatures of 5—7°C. The moths began to appear 11—15 days later, but the last ones only after 43 and 52 days respectively. On the other hand, the reactivation of animals kept in the warm after 2—3 months of cooling started earlier and was also completed earlier (Fig. 3). In another material kept at 23 and 28°C respectively without previous cooling moths appeared after maximum 111 and 99 days respectively, but several living caterpillars remained in diapause even after 172 days.

Knowing the critical time of illumination, the following can be established concerning the diapause conditions of populations developing in the open.

In the district of Keszthely the effective day-length (astronomical day-length plus one hour) decreases at the end of summer, between 20 August and 7 September, from 15 to 14 hours. According to 50 years meteorological data

(Bacsó 1959) at Keszthely the mean temperature in August is 20.6°C (at Szeged, the main zone of damage, 21.7°C), thus, it is the above period that must be considered as critical for the diapause of *G. delineana.*

From a practical point of view the above data indicate that in the period between 22 April and 20 August (if only the photoperiodical conditions are considered) generations can develop continuously, and that part of the population which develops after 20 August will show diapause to an increasing per cent. The smaller proportion which does not show diapause may start the development of a new generation, depending on the temperature conditions in September. This new generation will, however, show — with all certainty — a 100 per cent diapause.

Our experience of 10 per cent fresh pupae found outdoors in the southern part of Hungary even on 15th September is also in accordance with the above.

By beginning the harverst of fibre hemp earlier we can destroy a considerable part of the larva population. In Hungary fibre hemp is usually harvested in the middle of August. If the harvest takes place 7—10 days earlier, then at that time the bulk of the population will consist of partly developed caterpillars. After the harvest they leave the wilted drying plant parts and die. It may occur that more developed specimens complete their development in the green residues of the stubble field, especially if it is left unploughed. Therefore, the possibility of larva development is in close connection with the time of harvest. Harvesting at the end of August, beginning of September renders the development of the whole larva generation and its hibernation possible (Nagy 1967a, b).

The results of our diapause experiments also show that the suggested earlier harvest of fibre hemp makes it possible to destroy a considerable proportion of the larva progeny of the first summer generation before diapause sets in. By this the overwintering larva population can be essentially reduced.

Acknowledgement

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CHANGES OF GLYCOLIC ACID OXIDASE AND PEROXIDASE ACTIVITY IN MAIZE LEAVES DURING THE VEGETATION PERIOD

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Changes in the glycolic acid oxidase and peroxidase activity and in the chlorophyll content during the vegetation period were studied in the basal and apical parts of maize leaves. The glycolic acid oxidase activity was always found to be higher in the basal, younger parts of the leaf, nearer to the stem than in the farther apical tissues. The chlorophyll content, on the other hand, was higher in the older apical tissues. Parallel with the senescence of the leaves the glycolic acid oxidase activity decreased in each leaf horizon while the chlorophyll content did not essentially change. In the course of the stem formation the glycolic acid oxidase activity was much higher in leaves developed at the beginning of the growth season than in those developed in later phases, after flowering and pollination. On the basis of all these it has been found that during the development of the maize plant there is no close correlation between the glycolic acid oxidase activity and chlorophyll content of the leaves. Further it has been pointed out that it is not the age of the leaves but the physiological age of the plant that determines the extent of the glycolic acid oxidase activity. In the course of maize development the glycolic acid oxidase activity in the leaves showed a trend similar to the K- and opposite to the Ca-and NO_3—N content. On this basis certain correlation can be supposed to exist between the glycolic acid oxidase activity and the nutrient content of the leaves. A close correlation, parallelism between the age of the leaves and the extent of the peroxidase activity cannot always be found during the vegetation. Within the same maize leaf, again, it is not always the older tissue part that shows the higher peroxidase activity. Our further task is to study in detail the trend of the peroxidase level during the development of the maize plant, and disclose the causes of changes in its activity.

Introduction

In the metabolism of plants an essential role is played by the so-called end-oxidases, among others the glycolic acid oxidase and the peroxidase. The glycolic acid oxidase oxidizes the glycolic acid using the oxygen of the air whilst glyoxilic acid and H_2O_2 is either broken down to water and molecular oxygen by the catalase enzyme, or is used by the peroxidase for oxidizing certain reduced compounds, hydrogen donors. The glyoxilic acid produced may take part in the oxidization of the reduced pyridine nucleotides, may have a part in forming various compounds e. g. glycine, serine and phosphoglycerin acid (PRITCHARD *et. al.* 1962, RICHARDSON—TOLBERT 1961, TOLBERT—COHAN 1953, TOLBERT 1963, SCHOU *et al.* 1950). In addition it may contribute to the synthesis of proteins, carbo-hydrates and other compounds (KOLESNIKOV 1962a). The glycolic acid oxidase that takes part in the formation of the glyoxilic acid generally occurs in larger quantities in the green parts, leaves of the plants (Clagett *et al.* 1949. Kolesnikov 1948a, 1948b) and is strongly tied to the chloroplasts (Kolesnikov *et al.* 1970). When the leaves are infected or mechanically injured, e. g. with the isolation of leaves the chloroplasts and chlorophyll proteins begin to decompose, which process is accompanied by the decreased activity of the glycolic acid oxidase (Farkas 1963). In isolated leaves the decrease of glycolic acid oxidase and chlorophyll content can be prevented by placing the leaves in kinetin solution (Dézsi—Farkas 1964). The glycolic acid oxidase activity and the chlorophyll synthesis increase when the etiolated leaves are illuminated (Kolesnikov—Emenova 1956, Kuczmak—Tolbert 1962, Tolbert—Burris 1950). On the basis of these facts a close correlation is supposed to exist between the chlorophyll content and the glycolic acid oxidase activity of leaves. However, it has not been investigated so far whether in the leaves of plants raised under natural conditions a similar correlation exists during the vegetation period, and what trend the glycolic acid oxidase activity shows parallel to the development of the plant.

A characteristic concomitant of changes occurring in the metabolism of plants is the change of the peroxidase level. It is known that with an infection or mechanical injury of the plants the activity and isoenzyme composition of the peroxidase change (Bastin 1968, Farkas *et al.* 1964, Farkas—Stahmann 1966, Johnson—Cunningham 1972, Kanazawa *et al.* 1965, Loebenstein—Lindsey 1963, Lovrekovich *et al.* 1968, Novacky—Hampton 1967, Rubin—Arzichowskaya 1963). The enzyme activity changes when the seeds or leaves are treated with various elements and compounds (Suleymanov 1967, Dézsi *et al.* 1970). External, environmental conditions, illumination also change the peroxidase activity (Zenchenko 1964, Ivanova *et al.* 1970). The peroxidase activity increases with leaf senescence too (Novacky—Hampton 1968, Budilova *et al.* 1971). The question accordingly arises whether there is any difference in peroxidase activity between the younger and older leaf parts of the same plant, and what changes occur in the enzyme activity during the vegetation period.

In a previous experiment we found that in the course of maize development the K-content was always higher in the younger basal part of the leaf, while the Ca- and NO_3—N content in the older apical tissues (Dézsi—Frenyó 1969). This difference in nutrient content between the two parts of the leaf can supposedly be explained by a different metabolism. We may receive an answer to this question by studying the trend of the glycolic acid oxidase and peroxidase activity during the growth season.

Material and Method

Our investigations were made with the maize variety "Martonvásári 602" grown under outdoor conditions. Samples were taken for the analyses at the 9—11-leaf stage from the basal and apical parts of leaves of six uniformly developed plants on each occasion. The leaf parts were washed first in tap water then with distilled water, and 1 g of each sample was rubbed to pieces in a phosphate buffer. When determining the glycolic acid oxidase and the chlorophyll content a pH 7.8, while for the peroxidase determination a pH 6.0 phosphate buffer was used. The glycolic acid oxidase activity was determined by the colorimetric method of Kolesnikov (1962b) on the grounds of measuring the quantity of glyoxilic acid produced in a unit time. The chlorophyll content was determined after Arnon (1949). When determining the peroxidase activity the homogenizate was previously centrifuged for 10 minutes at 8000 rpm., and the enzyme activity was measured in the supernatant with guaiacol as a substrate by spectrophometer at 470 millimicron.

Results

In 1972 samples were taken on five occasions, and the basal and apical tissues of leaves from each leaf storey were separately analysed. We do not think it necessary to give here every detail of the investigations, but consider it essential to describe the vertical and horizontal distribution of the glycolic acid oxidase activity and chlorophyll content at two different dates (Table 1).

As seen in Table 1 the glycolic acid oxidase activity was always higher in the younger, basal parts of the leaves than in the older apical tissues. When comparing the enzyme activity of the lower and upper leaf storeys we find the former ones to be of lower value. On the basis of data obtained at the two different dates we can further see that with the senescing of leaves the glycolic acid oxidase activity decreases. In the case of the chlorophyll content we find an opposite trend, namely, it shows a higher value in the apical part of the leaves. There was no substantial difference between the chlorophyll contents determined at the two different dates. With the senescing of leaves the chlorophyll content slightly increased, in contrast with the decreasing activity of the glycolic acid oxidase.

To check our results we repeated the investigations in 1973. For the purpose of comparison we again give the detailed data of analyses performed at two different dates (Table 2). Table 2 shows that the glycolic acid oxidase activity (while in absolute value somewhat lower than in the previous year) was in this case too high in the younger basal part of the leaf than in the older apical tissue. In samples taken in an early phase of vegetation, at the time of flowering (18 July), the glycolic acid oxidase activity both in the basal and apical parts of the leaf far exceeded the values obtained in a later period of the growth season, at the time of grain formation (16 August).

As regards the chlorophyll content essential differences like those in the previous year were not found either between the basal and apical parts of the leaf, or between the lower and upper leaf storeys. The chlorophyll content of older leaves slightly increased in this case, too. It can thus be said that taking a longer interval of the growth season in consideration there is no close correlation between the glycolic acid oxidase activity and the chlorophyll content in the leaves. We do not naturally deny the possibility of such correlation

Table 1

Activity of glycolic acid oxidase and chlorophyll content trends in maize leaves in 1972

Time of sampling	Leaf storeys from below	Glycolic acid oxidase activity mg/g fresh weight		Chlorophyll content mg/g fresh weight	
		at the leaf base	at the leaf apex	at the leaf base	at the leaf apex
20 July	1	2.28	2.56	2.08	4.34
	2	3.96	2.88	3.60	4.05
	3	5.04	2.94	3.91	4.20
	4	5.76	3.24	3.91	2.95
	5	5.76	3.36	3.91	4.63
	6	7.20	4.44	3.91	4.63
	7	5.88	4.32	3.48	4.34
	8	7.02	3.96	3.60	4.20
	9	5.16	4.56	3.47	4.20
4 August	1	1.80	1.44	3.25	4.05
	2	2.52	1.38	3.50	4.92
	3	2.76	1.38	3.88	4.92
	4	2.94	1.50	4.63	4.63
	5	2.94	1.56	4.78	4.92
	6	3.30	2.10	4.78	4.92
	7	3.60	2.04	4.05	4.63
	8	3.72	2.76	3.71	4.63
	9	3.54	3.12	3.91	4.63
	10	3.48	3.30	3.91	4.63
	11	3.54	4.20	4.05	4.63

existing in certain cases — mainly in seedlings — at an early phase of vegetation.

The data of analyses showed that upwards from the fifth and sixth leaf storeys the glycilic acid oxidase activity was higher than in the lower leaves. Therefore the data obtained in the 1972 analyses of leaves from the lower and upper leaf storeys were summarized and the mean values included in Table 3. We have to note here that this distribution is justified by the fact that the spadix develops at the fifth to sixth leaf, so the trends of the glycolic acid oxidase activity and chlorophyll content in leaves below and above the spadix can be illustreted in a simpler way (Table 3).

The data of Table 3 clearly show the great change the glycolic acid oxidase activity undergoes during the growth season. In an early phase of the vegetation period, at the time of stem formation (7 July) the glycolic acid oxidase activity was five to eight times higher than at the latest date of examination:

Table 2

Glycolic acid oxidase activity and chlorophyll content trends in maize leaves in 1973

Time of sampling	Leaf storeys from below	Glycolic acid oxidase activity mg/g fresh weight/h		Chlorophyll content mg/g fresh weight	
		at the leaf base	at the leaf apex	at the leaf base	at the leaf apex
18 July	1	1.62	1.50	3.55	3.84
	2	2.64	1.92	3.75	3.90
	3	2.46	1.92	3.75	3.90
	4	2.52	2.34	3.92	4.19
	5	3.48	2.28	3.95	4.19
	6	3.12	2.52	4.01	4.24
	7	3.78	2.82	3.92	4.22
	8	4.32	2.82	3.92	4.05
	9	4.92	4.08	3.75	3.95
	10	5.76	4.62	3.75	4.05
16 August	1	1.08	0.90	3.72	4.13
	2	1.44	1.14	4.05	4.48
	3	1.53	0.90	4.10	4.56
	4	1.83	1.47	4.33	4.77
	5	2.28	1.26	4.63	5.00
	6	2.01	1.11	4.77	5.06
	7	1.74	1.08	4.63	4.98
	8	1.74	0.90	4.69	5.15
	9	1.83	1.47	4.63	5.12
	10	1.50	1.20	4.42	5.00
	11	1.80	1.35	4.40	4.98

at waxen ripening (31 August). It is known from the literature that with the senescing of leaves the glycolic acid oxidase activity decreases (FARKAS 1968). However, in our present investigation we have to take it in consideration that the upper leaves of the maize plant develop in a later period of the growth season and are thus relatively young, still they show a comparatively low enzyme level. Looking at the detailed data of Table 1 we find that the enzyme activity of each leaf storey was much higher on 20th July than two weeks later, on 4th August. In spite of the fact that the leaves of the uppermost storey developed during these two weeks (10th to 11th leaf storey) and were thus the youngest of all at that time, still the value of their glycolic acid oxidase activity corresponded — with a single exception — to that of the lower older leaves. At the beginning of the growth season no such change was observed. So e. g. on 7th July, that is at the 5-leaf stage, the average enzyme activity

Table 3

Glycolic acid oxidase activity and chlorophyll content in the lower and upper leaf storeys

Time of sampling	ycolic acid oxidase activity mg/g fresh weight/hour				Chlorophyll content mg/g fresh weight			
	average of the lower 5 leaves		average of the upper leaves		average of the lower 5 leaves		average of the upper leaves	
	base	apex	base	apex	base	apex	base	apex
1972. 7 July	6.29	5.47			3.03	4.10		
20 July	4.56	3.00	6.31	4.32	3.48	4.03	3.61	4.34
4 August	2.60	1.45	3.56	2.92	4.01	4.69	4.07	4.63
16 August	0.90	0.57	1.26	0.75	3.83	4.38	4.04	4.41
31 August	0.80	0.73	1.33	0.62	3.66	3.82	4.14	4.13

in the basal parts of the leaves was 6.29 mg/g fresh weight/hour; two weeks later, on 20th July in the basal parts of the newly developed leaves of the upper storey a 6.31 value was obtained. The glycolic acid oxidase activity in the lower five leaves was at the same time 4.56 mg/g fresh weight/hour, which shows a considerable decrease of enzyme activity. Thus the glycolic acid oxidase activity of the younger leaves exceeded the enzyme activity of the older leaves by some 40 per cent. In a later period of the growth season — as referred to before — this did not occur. On the basis of these facts we can say that in a later phase of the ontogenesis of maize the extent of glycolic acid oxidase activity is determined by the physiological age of the plant rather than by the age of the individual leaves.

In connection with our data we should like to call attention to another factor. Few studies have been performed so far as to whether the nutrient supply of plants influences — and if so in what form — the activity of the glycolic acid oxidase. Zaytseva *et al.* (1971) pointed out that with the phosphor deficiency of plants the glycolic acid oxidase activity increased. As regards other nutritive elements we also found certain correlations. In the same maize variety as used in the present experiment we examined the distribution of nutritive elements in the leaves during the vegetation period (Dézsi—Frenyó 1969). We found the potassium content to be always higher in the basal parts than in the apical parts of the leaves. In the course of the vegetation period the potassium content gradually decreased both in the basal and apical parts. The calcium content showed an opposite trend. The quantity of NO$_3$—N was always lower in the basal part of the leaf than in the apical tissues suggesting a more intensive metabolism in the younger parts of the leaves. With the senescing of the plants the NO$_3$—N content of the leaf increased both in the basal and apical parts suggesting a declining metabolic activity. Comparing these data with the results of our present experiment we can say that the

Table 4

Peroxidase activity in maize leaves

Time of sampling	Leaf storeys from below	Extinction values measured by spectrophotometer		
		at the base	at the apex	base/apex %
1973	1	465	385	80
13 July	2	405	442	109
	3	412	465	112
	4	380	395	104
	5	495	515	104
	6	412	405	99
	7	415	520	79
	8	375	415	110
	9	280	385	128
	10	285	355	120
	11	285	372	124
23 July	1	342	292	84
	2	320	270	82
	3	342	330	97
	4	360	345	96
	5	385	325	82
	6	367	295	76
	7	310	292	94
	8	310	382	119
	9	350	312	88
	10	360	312	85
	11	290	260	89
13 August	1	512	397	72
	2	540	500	92
	3	610	432	59
	4	527	585	110
	5	640	537	81
	6	572	465	77
	7	572	480	81
	8	650	465	61
	9	510	455	88
	10	675	515	69
	11	650	470	62

glycolic acid oxidase activity of the maize leaf shows a similar trend to the K- and opposite trend to the Ca- and NO_3—N contents. This suggests a possible correlation between the nutrient content and the glycolic acid oxidase activity of the leaves.

We examined the change of activity of another oxidative enzyme — peroxidase — in maize leaves. The relevant data are presented in Table 4. As seen from the figures of the table there is, in general, no such great difference in peroxidase activity between the leaf base and leaf apex as in the case of glycolic acid oxidase. It can be said that in the early phase of the growth season it was in the apical part, that is in the older tissues, while later in the younger basal part that the peroxidase activity was somewhat higher. No substantial difference in peroxidase activity was found between the lower, older, and upper, relatively younger leaves either. On the basis of the literature we expected the peroxidase activity to increase with the senescence of the plant, that is, we thought to find a graduality in the distribution of the peroxidase level of maize leaves both horizontally and vertically. Our data did not, however, confirm this parallelism, they even showed a considerable fluctuation. The cause of this fluctuation, of the uneven distribution of the peroxidase level is not naturally known as yet. It may be caused by climatic factors, or by the alternation of the development phases of plants; it is the task of the future to throw light upon this problem.

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EFFECT OF GUANIDINO-METHYLATED ARGININES ON THE GROWTH OF TOBACCO TISSUE CULTURES

By

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Studying the effect of L-arginine and its guanidino-methylated derivatives on tobacco tissue cultures the authors found that 10 and 100 ppm concentrations of MMA (N^G-monomethyl-L-arginine) and DMA' (N^G, N'^G-dimethyl-L-arginine) caused 20—80 per cent growth inhibition in 41 and 62 days old cultures, respectively. The added L-arginine and the added two guanidino-methylated arginines could be detected by layer chromatography in alcoholic extracts of both the tissue cultures and the culture medium. Since the guanidino-methylated arginines are not readily demethylated it is supposed that they influence the lysine-arginine antagonism thus forming a part of the system of natural growth regulation.

Introduction

Among the protein-amino acids of living organisms L-arginine plays an important role — even according to the limited knowledge acquired so far.

The synthesis in cell cultures of various adenoviruses is only possible in the presence of arginine (Rouse—Schlesinger 1967, Dubes *et al.* 1969). In the absence of arginine the virus DNA and the capside antigens are synthetized in a reduced yield in the infected cells, but non infectious viruses can be detected in every cell. If arginine is then added to these cells, there is a rapid increase in infectivity. Arginine is needed for the replication of the herpes virus too (Inglis 1968).

Arginine is similarly indispensable in culturing microorganisms (Chang—Shung 1968, Pass—Razynska-Bojanowska 1969, Kuz'menko 1969).

In the metabolism of plants arginine serves as a nitrogen source in the biosynthetic processes whereas — as an acceptor — it is one of the main binders of nitrogen (Pléskov—Vil'yams 1965, Baret *et al.* 1966). In some species of *Leguminosae* and in those belonging to the *Cucurbitaceae* intensive arginine metabolism takes place especially during germination (Jones—Boulter 1968). It is remarkable that the stress effects are particularly felt on the arginine level of plants. E. g. in the young leaves of coconut-palms diseases considerably increase the arginine content (Pillai—Shanta 1968). In sugar-beet leaves too the arginine level gives an intensive growth response to virus infections (Lerch—Stegemann 1970).

In the animal organism, arginine as a member of the urea cycle, by removing the ammonia or ammonium ions promotes the detoxication of the organism (ROBERGE—CHARBONNEAU 1969). The physiological importance (essential role) of arginine intake has been pointed out in the case of a number of animals (GRADUSOV 1967, ROGERS *et al.* 1970). It has been generally proved that the addition of excess L-lysine to the diet of young chicks increases the requirement for arginine, which appears in a decreased rate of growth, symptoms of arginine deficiency, as well as in the lower arginine level of the plasma too (JONES 1964, O'DELL—SAVAGE 1966, BOORMAN—FISHER 1966). The decreased arginine level of the plasma is probably caused by the absorptive loss of arginine on the renal tubules of the chick resulting from a competition between lysine and arginine (JONES *et al.* 1967).

The lysine-arginine antagonism can be particularly well demonstrated by their effects exerted on tumours in animals. L-lysine proved to have a considerable retarding, while L-arginine a promoting effect on tumour growth, while the corresponding antipodes showed the opposite trend (TYIHÁK—SZENDE 1967—1970). On the basis of protein synthesis investigations made on animal tumours GRAFFI *et al.* (1965) suggested reducing the quantity of arginine given to cancer patients to a minimum, and thought it important to prevent the incorporation of arginine in proteins by using various natural antagonists (ornitine, citrulline, glycine, etc.).

The influence of arginine on growth — like the study of other bioactive substances — can be efficiently studied in plant tissue cultures. For example in suspension cultures of sugarcane cells (NICKELL—MARETZKI 1969, MARETZKI *et al.* 1969), as well as in callus tissue cultures of rice (FURUHASHI—YATAZAWA 1970) arginine was proved to have an important role in promoting growth.

Guanidino-methylated derivatives of arginine N^G—monomethyl-L-arginine (MMA), N^G, N^G-dimethyl-L-arginine (DMA) and N^G, N'^G-dimethyl-L-arginine (DMA') have recently been detected in some animal (PAIK—KIM 1970, 1971, BALDWIN—CARNEGIE 1971) and plant proteins (TYIHÁK 1972—1973) as well as in a free state in various tissues and biological liquors (KAKIMOTO—AKAZAWA 1970, TYIHÁK—PATTHY 1973). The role of these natural amino acids in living organisms has not been cleared up so far. On the ground of the well-known growth promoting effect of arginine (SCHWERDTFEGER 1971), as well as of the tumour growth promoting (SZENDE *et al.* 1970, KOPPER *et al.* 1971), and general growth promoting effect (TYIHÁK *et al.* 1971) of ε-N-methylated lysines we supposed the guanidino-methylated arginines to have an inhibitory effect on growth.

To support our theory we carried on preliminary studies concerning the effect exerted by L-arginine and its guanidino-methylated derivatives on the growth of tobacco tissue cultures.

Material and Method

The test material used in our investigations was a secondary callus tissue isolated from a tobacco (*Nicotiana tabacum* L.) stem. The tissue consisted of a yellowish-green cell population, was of intensive growth, and on a standard culture medium did not show organ formation only some tissue differentiation.

Of the amino acids used L-arginine was a commercial product (Reanal Chemical Works, Budapest), while the guanidino-methylated L-arginine derivatives were prepared by synthesis (Bajusz—Patthy 1972).

The aqueous solution of amino acids to be examined was sterilized by a Seitz-filter and added in sterile state to an agar-agar culture medium not yet solidified (Maróti 1969). The different amino acids were applied at concentrations of 1.0—10.0—100.0 mg/l culture medium. In the Erlenmeyer dishes each piece of tissue was placed on 50 ml culture medium, with an initial weight of 200 mg. From the variants two parallel experiments were set up with four replications each. The tissues grew for 41 and 62 days, respectively, in a thermostat of 28 ($\pm$2)°C temperature with a natural alternation of day and night.

To control the cell-morphological effect of arginine and its guanidino-methylated derivatives we measured the fresh weight of the variants, and calculated the daily growth rate (end weight—initial weight/number of days) and the relative growth value (end weight — initial weight/initial weight).

Study on the free amino acids of the culture medium and tobacco tissue. The culture medium or tissue samples were first reduced to pulp with a double amount of 96 per cent alcohol in a Waring blendor, then the suspension was centrifuged at 3000 rpm. The supernatant was decanted; then the culture medium- or tissue pulp was washed again with a single dose of alcohol, and centrifuged. The supernatants were joined, and definite quantities of solutions of the individual samples dropped into Fixion 50 × 8 (Chinoin Nagytétény Factory, Budapest) ion exchange containing chromatosheets previously equilibrated with sodium citrate buffer (pH 3.28, 0.02 n Na^+). The eluting buffers used in the procedure were: a) 1 n acetic acid — 1 n Na-acetate and 1 n NaCl at a ratio of 1 : 1 : 1; b) 50 g citric acid + 30 g NaOH + 7 ml cc. HCl in 500 ml distilled water (Tyihák *et al.* 1974), and room temperature was maintained during the operation. The amino acids were developed with an acetonic ninhydrin reagent (0.5 g ninhydrin + 0.05 g $CuSO_4 \cdot 5\ H_2O$ in 100 ml aceton) by warming at 70°C for 10 minutes.

Determination of the total nucleic acid and total protein contents of tobacco tissues. Alcohol extracted and dried tobacco tissue samples of 75 mg weight were pulpified with 5 ml cold (0°C) TCA of 5 per cent concentration for 20—30 seconds, then allowed to stand for 30 minutes at 0°C. After that the solutions were centrifuged cold (0°C) at 7500 rpm., the supernatants decanted, then the tissue residues treated with a further 5 ml TCA in the above way and the supernatants decanted again. 5 ml 5 per cent concentration TCA was poured on each tissue residue, the mixture pulpified for 20 minutes, then the solutions were heated for 20 minutes in a 90°C water-bath. When cooled the solutions were centrifuged at 7000 rpm., and the supernatants photometried by means of a Spektromom 201 spectrophotometer at 268.5 nm. 5 ml 1 n NaOH solution was poured on each of the tissue residues which were then pulpified for a short time (30 seconds) and hydrolysed over 48 hours at 37°C. The solutions were centrifuged at 5000 rpm. then the supernatant evaluated by spectrophotometer at 260 and 280 nm.

Results

As seen from Figs 1 and 2 at concentrations of 10 and 100 ppm MMA and DMA' respectively, considerable growth inhibition could be attained after 62 days of culturing compared to the control. And Fig. 3 shows that after 62 days, with the highest (100 ppm) dose applied, L-arginine also displayed growth inhibition, but MMA and DMA', in the case of the same dose, showed a significant inhibition even compared with the arginine.

Table 1 presents detailed data. A comparison between the end weights determined after 41 and 62 days of culturing, respectively, reveals that growth

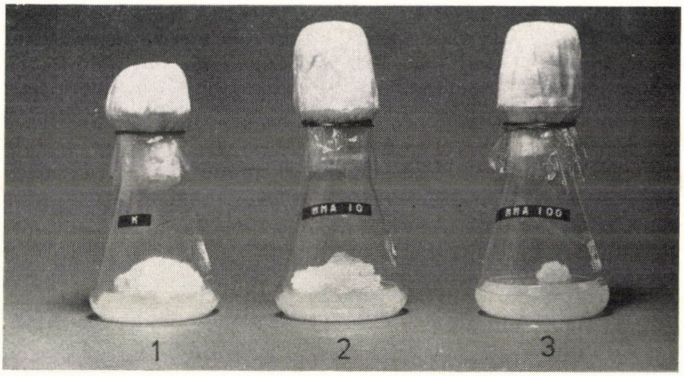

Fig. 1. Effect of MMA (N^{G}-monomethyl-L-arginine acetate) on the growth of tobacco tissue cultures. (1 = control; 2 = 10 ppm MMA; 3 = 100 ppm MMA)

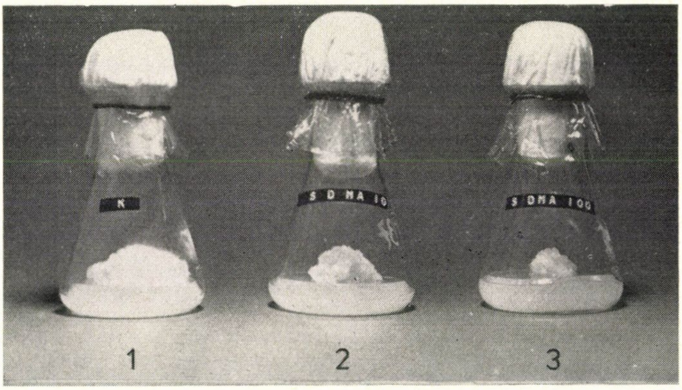

Fig. 2. Effect of DMA' (N^{G}, N'^{G}-dimethyl-L-arginine HCl) on the growth of tobacco tissue cultures. (1 = control; 2 = 10 ppm DMA'; 3 = 100 ppm DMA')

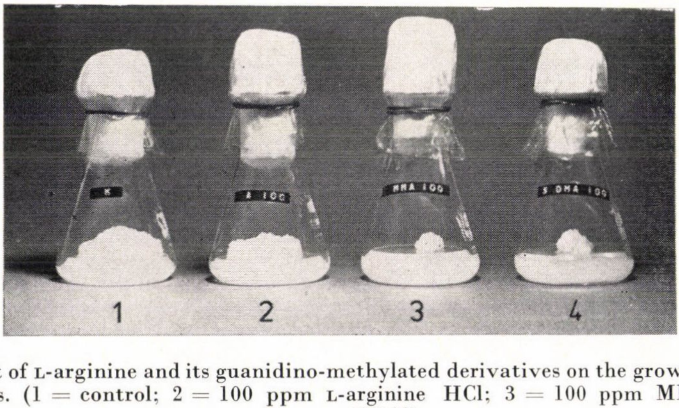

Fig. 3. Effect of L-arginine and its guanidino-methylated derivatives on the growth of tobacco tissue cultures. (1 = control; 2 = 100 ppm L-arginine HCl; 3 = 100 ppm MMA; 4 = 100 ppm DMA')

Table 1

Effect of arginine and its guanidino-methylated derivatives on the growth of tobacco tissue cultures

Treatment	End weight		Daily growth		Growth inhibition (−) stimulation (+)	
	41 days	62 days	41 days	62 days	41 days	62 days
	g		mg		%	
Control	4.776	16.38	112.9	264	0	0
A-1	4.264	—	101.6	—	−11	—
A-10	4.574	16.22	107.8	261	− 5	− 1
A-100	3.895	8.49	90.0	137	−19	−49
MMA-1	4.507	—	107.6	—	− 6	—
MMA-10	2.852	9.88	66.3	159	−41 ($P_{5\%}$)	−40 ($P_{1\%}$)
MMA-100	1.649	2.32	35.3	37	−66 ($P_{1\%}$)	−87 ($P_{0.1\%}$)
DMA'-1	5.484	—	132.1	—	+14	—
DMA'-10	3.578	10.23	84.4	160	−26 ($P_{5\%}$)	−38 ($P_{1\%}$)
DMA'-100	3.524	3.84	81.0	62	−27 ($P_{5\%}$)	−77 ($P_{0.1\%}$)

Signs and abbreviations:

A = L-arginine; MMA = N^G-monomethyl-L-arginine; DMA' = N^G, N'^G-dimethyl-L-arginine

1 = 1 mg amino acid/lit culture liquid
10 = 10 mg amino acid/lit culture liquid
100 = 100 mg amino acid/lit culture liquid

inhibition by the guanidino-methylated arginines as compared to the control and the arginine is more expressed in the 62 days than in the 41 days culture.

As seen from Fig. 4 amino acids added to tobacco tissue cultures grown on a culture medium containing the amino acids in question (Arg, MMA, DMA') at a concentration of 100 ppm can be pointed out and identified in the alcoholic extract, and DMA' can be found in the corresponding extract in particularly large quantities. Fig. 5 — where a higher amount of extract was poured on in drops — further shows that compared to the control extract the added amino acid can be seen even in the DMA'—10 (10ppm) extract. This figure makes it even clearer that the three amino acids show the following order of enrichment in the tissue compared to the control: control$<$arginine MMA$<$DMA' (ninhydrine sensitivity shows a reverse order !). Fig. 6 shows that arginine, MMA and DMA' can be demonstrated by layer chromatography even in alcoholic extracts of culture media distant from the tissue, and that almost in the same quantities as in the tissue extracts.

After 41 and 62 days the total nucleic acid and protein content of the tissues was also determined, but differences unequivocally accounting for the growth inhibition were not obtained.

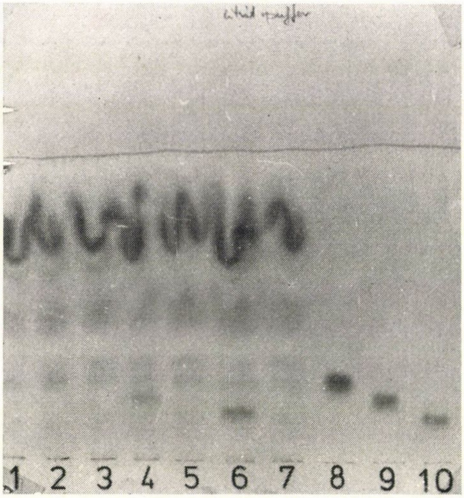

Fig. 4. Examination of alcoholic tobacco tissue extracts on Fixion 50×8 chromato sheet (b/buffer). (1 = 10 ppm L-arginine HCl; 2 = 100 ppm L-arginine HCl; 3 = 10 ppm MMA; 4 = 100 ppm MMA; 5 = 10 ppm DMA'; 6 = 100 ppm DMA'; 7 = control; 8 = L-arginine HCl; 9 = MMA; 10 = DMA'. Of extracts: 0.02 ml each; of test material: 2 μg each)

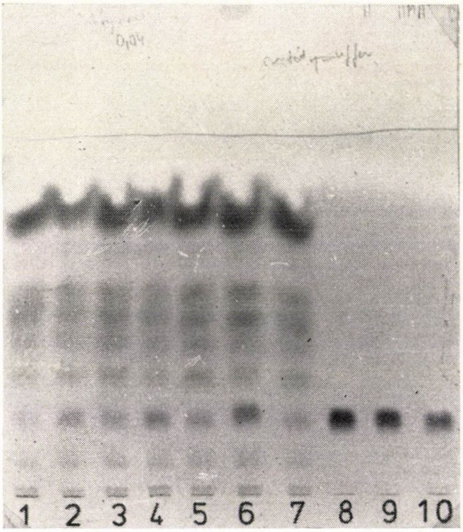

Fig. 5. Examination of alcoholic tobacco tissue extracts on Fixion 50×8 chromato sheet (a/buffer). The order of the samples is the same as in Fig. 4. The amount of extract used: 0.04 ml

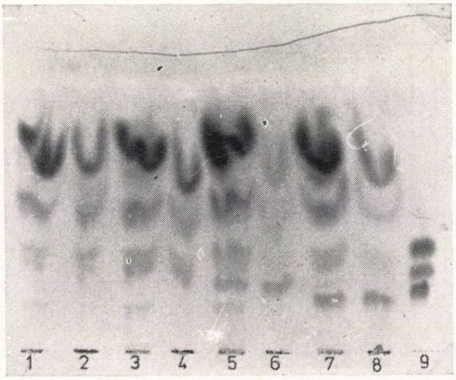

Fig. 6. Examination of alcoholic tobacco tissue and culture medium extracts on Fixion 50×8 chromato sheet (b/buffer). (1 = control (tissue); 2 = control (culture medium); 3 = L-arginine HCl 100 ppm (tissue); 4 = L-arginine HCl 100 ppm (culture medium); 5 = MMA 100 ppm (tissue); 6 = MMA 100 ppm (culture medium); 7 = DMA' 100 ppm (tissue); 8 = DMA' 100 ppm (culture medium); 9 = amino acid tests: L-arginine, MMA and DMA'. Amounts applied: of tissue extracts 0.05 ml, of culture medium extracts 0.075 ml)

Discussion

With our investigations made on tobacco tissue cultures — under the given experimental conditions — we succeeded in confirming our theory concerning the growth inhibitory effect of guanidino-methylated arginines.

The results of our investigations prove that in the case of treatments performed with guanidino-methylated arginines a permanent inhibition of arginine incorporation, of vital importance for the tobacco tissue too, results in growth inhibition. The latter statement seems to be supported by the results of layer chromatographic examinations carried out with the free amino acids of the culture medium and the tissue, according to which the guanidino-methylated arginines do not decompose even after 62 days in the culture medium, and the tobacco tissue practically cannot break down the guanidino-methylated arginines (mainly the DMA').

Although from the fact that a relatively large amount of guanidino-methylated arginine accumulates a day in the human urine too (Kakimoto—Akazawa 1970) no general conclusion can be drawn, but it can be imagined that the guanidino-methylated arginines — as they are not readily demethylated — influence the lysine-arginine antagonism and so form a part of the natural growth regulatory system. In this sense the results of our investigations made with guanidino-methylated arginines on tobacco tissue may serve as additional material to the "retine-promine" question (Tyihák 1972, Tyihák—Patthy 1973).

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STUDY ON THE EFFECTS OF ECOLOGICAL FACTORS ON SOLANUM DULCAMARA

By

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The authors studied the habitat dependent productivity of plant stands propagated vegetatively from a *Solanum dulcamara* L. taxon containing solasodine and soladulcidine besides tomatidenol as the main aglycon. They found that both the plant weight and the alkaloid production were highly influenced by the ecological factors. They attempted to express this effect of the ecological factors (first of all precipitation and soil) on productivity in a way suitable for comparison.

Introduction

Investigations into the chemical composition and geographical distribution of *Solanum dulcamara* revealed that on the basis of the steroid alkaloids tomatidenol, solasodine and soladulcidine occurring in the plants three chemotaxa can be distinguished (ALKEMEYER—SANDER 1959, BOGNÁR—MAKLEIT 1965, ROZUMEK—SANDER 1962, DERSCH—SANDER 1962, SANDER 1963b SCHREIBER—RÖNSCH 1963). The designation of the taxa named after the above alkaloids refers to the components occurring in the largest quantity in the respective plants. RÖNSCH *et al.* (1965), SCHREIBER—RÖNSCH (1965), WILLUHN (1966) give an account of taxa containing the above components in a pure state.

Our investigations made so far (MAKLEIT *et al.* 1967, MÁTHÉ —MÁTHÉ Jr. 1970, MÁTHÉ Jr. 1970, MÁTHÉ—MÁTHÉ Jr. 1972) suggest that taxa containing a single component — if they exist at all — are very rare. Although *Solanum dulcamara* can be far better characterized by the occurrence of more than one alkaloid, a distinction is justified among the different chemotaxa on the basis of the component found in the highest quantity. In the course of fruit analyses BOLL—ANDERSEN (1962), too, pointed out the simultaneous occurrence of the individual components.

Studies — partly of ecological nature — carried out so far revealed certain differences among the taxa found in various habitats (BOGNÁR—MAKLEIT 1965, ROZUMEK—SANDER 1967, SANDER 1963b, MÁTHÉ—MÁTHÉ Jr. 1972). Besides establishing the differences due to the geographical distribution the authors have arrived at the conclusion that the chemotaxa are genetically fixed as for their chemical character, so the latter can be considered a property independent of the ecological effects. The examinations have further disclosed (MÁTHÉ—MÁTHÉ Jr. 1970, MÁTHÉ Jr. 1970, MÁTHÉ—MÁTHÉ Jr. 1972) that the alkaloid contents of the individual plant organs vary to a great extent according to the development stage and ecological conditions of the plant. Beyond the alkaloid content the ecological factors naturally exercise a considerable influence on the quantity of plant organs, too. From among the ecological factors the effects of the habitat — more closely: that of the soil — on *Solanum dulcamara* plants will be discussed in this paper.

Material and Method

For our comparative studies we produced two stands from the same plant by vegetative propagation on 28th May 1971. Our plants provided a material roughly of identical value containing solasodine and soladulcidine beside the main aglycon tomatidenol. The two stands were planted close to each other (at a distance of about 30—40 m), but in soils of different character; this way similar meteorological and even microclimatic conditions were ensured. The major soil properties of the two sites are shown in Table 1, data on the depths of 0—10 and 10—20 cm are given separately.

Table 1

*Soil data of the A- and B-plots of Solanum dulcamara**

Basic analytical data	A-habitat sandy soil 0—10 cm	A-habitat sandy soil 10—20 cm	B-habitat heavy soil 0—10 cm	B-habitat heavy soil 10—20 cm
pH in water	7.5	7.6	8.0	8.0
pH n KCl	6.9	6.8	7.7	7.7
y_1	1.0	1.0	0.7	0.7
$CaCO_3$ %	traces	traces	6.5	7.1
humus %	1.5	1.5	5.6	4.8
Q	1.939	2.500	4.458	6.731
N %	0.021	0.018	0.084	0.072
NH_3—N mg/100 g	0.2	0.2	0.3	0.4
NO_3—N mg/100 g	0.2	0.6	4.4	3.3
K_2O mg/100 g	20.5	9.4	7.3	6.3
P_2O_5 mg/100 g	28.6	30.0	17.0	4.4

* We thank Dr. M. Kovács for the soil analysis.

As seen from the table, in plot B) the amounts of $CaCO_3$, humus and nitrogen were essentially larger than in plot A). In the latter the quantities of potassium and phosphorus were higher. Thus, plot B) can be simply characterized as a habitat of heavier humous soil, while plot A) as one with a light sandy soil. Since we have found (MÁTHÉ—MÁTHÉ Jr. 1972) that the water contents of the soil also has an important role we complete the characterization of the habitats by presenting the amount of precipitation from April to the harvest of the plants (Table 2).

Table 2

Precipitation (mm) on the experimental area at Vácrátót from the beginning of the vegetation period until collection for analysis

	1971	1972
April	34.9	115.8
May	114.6	159.5
June	77.5	86.0
July	56.0	83.9
Total	283.0	445.2

For the evaluation of the ecological effects we collected drugs from both plots on 18th July 1971 and 21st July 1972. We processed 15 plants from each plot by weighing the fresh weight of the different organs, measuring the total length of the shoots and — after drying at 70°C — weighing the dry weight of the organs. We performed a titrimetric determination of the alkaloid content in each organ of each plant. Titration was carried out with p-toluol-sulphonic acid in chloroform, in the presence of dimethyl-yellow as indicator, after a low concentration acidic extraction and acidic hydrolysis of the analytically measured quantity of the drug. Details of our procedure were given earlier (MÁTHÉ Jr. 1970).

Results

The effect exerted by the habitat on the plant organs was studied on second year plants collected on 21st July 1972. Tables 3 and 4 show the average data of the organs of 15 plants from each of plots A) and B).

The quantity of the plant organs decreased, independently of the habitat, in both plots in the following order: stem, leaf, green fruit, root, ripe fruit, as shown by either the fresh or the dry weights. When comparing the two habitats we found plot B) superior to plot A) in both the quantity of organs

Table 3

Average yield data of plants from plot A) in 1972

Plant parts	Weight (g)		Alkaloid	
	fresh	dry	%	total (mg)
Leaf	108 ± 35	25 ± 8	0.88	220
Stem	218 ± 88	76 ± 31	0.19	144
Green fruit	100 ± 61	13 ± 21	0.57	74
Ripe fruit	21 ± 8	5 ± 2	0.31	15
Root		27 ± 10	0.18	49
Total		146 ± 50		502

and the total weight of plants except the ripe fruit. Taking the percentage distribution of the dry weight among the plant organs (Fig. 1) into consideration we have found no substantial difference between the two habitats; thus, the differences in weight production between the habitats appear proportionately in the plant organs. The fresh weight data — while only referring to the above-ground parts — show a higher differentiation concerning distribution of the plant organs. This is expressed by the ratio of fresh weight-dry weight, too. While in the order of leaf, stem, green fruit, ripe fruit this ratio was 5.1; 3.6; 5.9; 4.0 in plot B), it was 4.3; 2.9; 7.7; 4.2 in plot A); that is, with the leaf and stem the ratio of desiccation was much greater in plot B) than in plot A), while in the case of the green fruit it was the other way round. As seen from the above the green fruit contains an essentially larger amount of water than the ripe fruit.

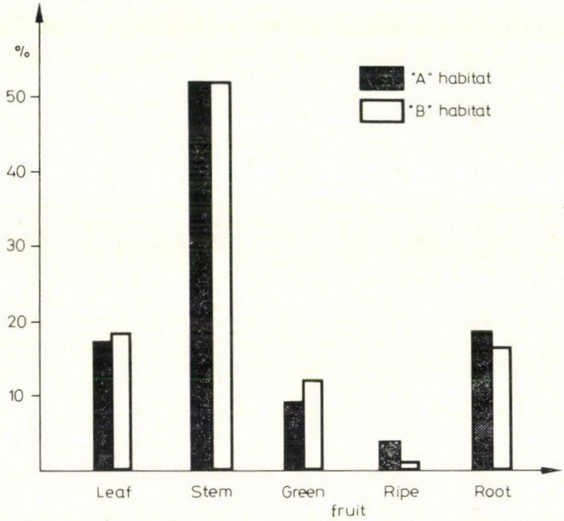

Fig. 1. Percentage distribution of dry weights of *Solanum dulcamara* plant organs

Tables 3 and 4 show the percentage and total alkaloid contents of the individual organs. The percentage proportion of alkaloid contents to total dry weight decreases as follows: leaf, green fruit, ripe fruit, root, stem. In the latter two organs the values are roughly the same. If, on the other hand, we consider the total alkaloid contents in the different organs we find that the highest quantity of alkaloid was produced by the leaves in both plots; but while in plot A) the decrease of the alkaloid contents in the other organs showed the following order: stem, green fruit, root, dry fruit, in plot B) the order of decrease was: green fruit, stem, root, dry fruit. The difference in the order of alkaloid production by the stem and green fruit can be traced back

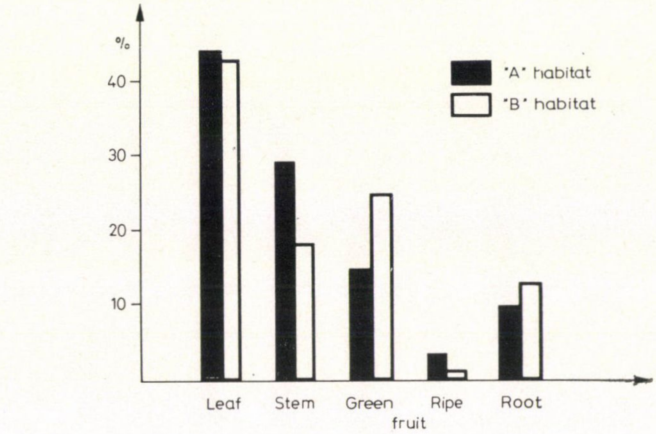

Fig. 2. Percentage distribution of the total alkaloid content of *Solanum dulcamara* plant organs

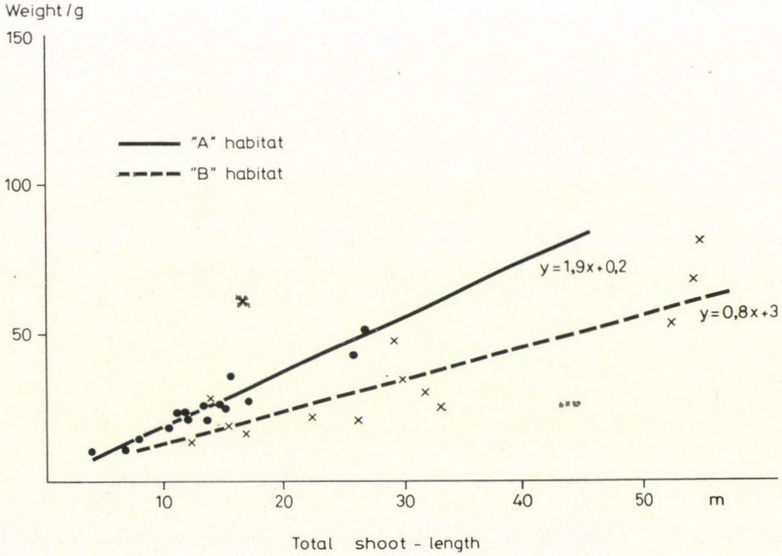

Fig. 3. Dry weight of leaf as a function of the total shoot-length per plant

to a difference in the total quantity of these organs in the two habitats. The percentage distribution per plant of the alkaloid content of the individual organs is shown in Fig. 2.

In Tables 3 and 4 the variance of weight production indicates that even the material originating from the same plot was highly variant. Omitting the presentation of measuring data concerning the individual plants we have plotted the dry weights of the different plant organs as a function of the total length of the shoots (Figs 3—5). (The average total shoot-lengths per plant

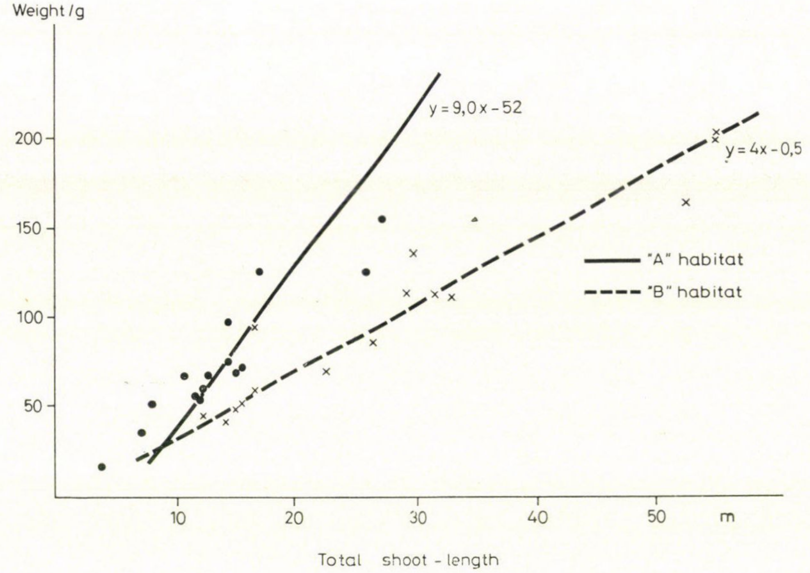

Fig. 4. Dry weight of stem as a function of the total shoot-length per plant

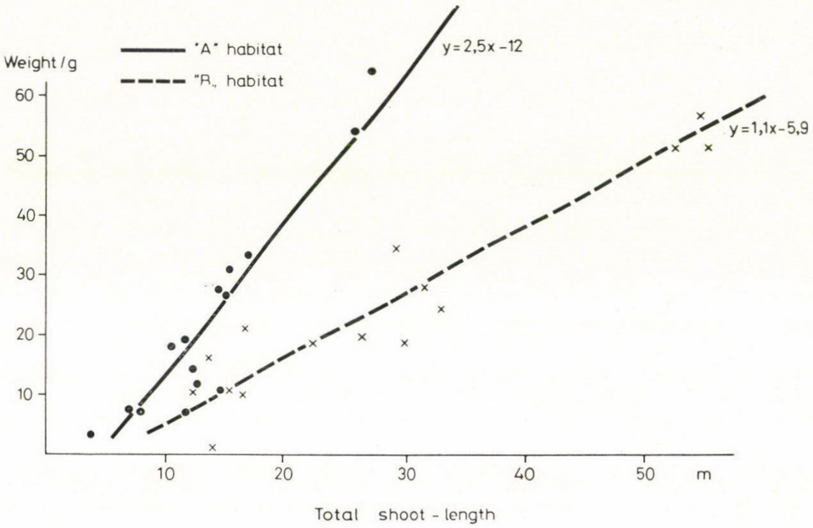

Fig. 5. Dry weight of green fruit as a function of the total shoot-length per plant

in plot A) and B) are contained in Table 5.) The figures reveal that in plot A) the dry weights of leaf, stem and green fruit as a function of the total length of the shoots varied — increased — more rapidly than in plot B) — as also shown by the regression coefficients of the regression equations expressing the changes. The quotients of the regression coefficients of leaf, stem and

Table 4

Average yield data of plants from plot B) in 1972

Plant parts	Weight (g)		Alkaloid	
	fresh	dry	%	total (mg)
Leaf	184 ± 99	36 ± 20	0.74	266
Stem	363 ± 182	102 ± 51	0.11	112
Green fruit	142 ± 93	24 ± 19	0.65	156
Ripe fruit	8 ± 7	2 ± 1	0.33	7
Root		32 ± 9	0.25	80
Total		196 ± 85		621

Table 5

Yield data of Solanum dulcamara stands in two different habitats

	Year of collection	
	1971	1972
Total length of shoots (m)		
Plot A)	2.7	14
Plot B)	2.2	28
Total above-ground dry weight (g)		
Plot A)	13	119
Plot B)	32	164
Total above-ground alkaloid content (mg)		
Plot A)	60	453
Plot B)	191	541

green fruit for plots A) and B) are 2.38, 2.25 and 2.27, that is, the values characteristic of the individual organs are nearly the same. As for the change in production, the second-year plants — in 1972 — were found to be more favourable in plot A) in spite of the fact that the average values of weight were higher in plot B). To understand the above apparent contradiction we have to examine the effects of the habitats A) and B) under the climatic conditions of the year 1971. In Table 5 some average data on the production of first-year plants are compared with the corresponding data of 1972.

In this table the data of the average total shoot-length per plant, the total dry weight of the above-ground parts and the amount of alkaloid contained in them show a substantial growth in 1972 compared to the first year stand for both plots. The table further reveals that the production of plot B) was higher in both years. The difference between the effects of the two habitats, that is, between the production results of the two plots, showed in 1972 a decreasing tendency compared to 1971. Expressing the changes in the dry weights of the above-ground parts as a function of the per plant total shoot-length of the 15 samples taken from each of the two plots for the analyses, the correlations can be determined by the following regression equations:

In 1971 A) habitat $y = 4.4\times + 1.6$
B) habitat $y = 10.0\times + 3.9$

In 1972 A) habitat $y = 10.3\times + 13.4$
B) habitat $y = 5.5\times + 10.0$

As seen from the above in 1971 the regression coefficients had a higher value in plot B), while in 1972 in plot A), which seems to express that under the climatic conditions of the year 1971 the ecological factors of plot B), while in 1972 those of plot A) were more favourable for the productivity of plants, as suggested also by Table 5. Thus the regression coefficients seem to be suitable for evaluating the year-by-year changes of the ecological effects exerted on the perennial *Solanum dulcamara*, therefore, we think it reasonable to express the changes in the quantity of the individual plant organs, or in the total amount of the above-ground parts, as a function of the total length of the shoots developed in the given year from plants previously cut back, and characterize the productivity of the habitat in the given year with the regression coefficient of the linear correlation thus obtained. The differences between these regression coefficients (expressing, in fact, the change of weight production per unit shoot-length) — during our investigations — show the differences between the ecological effects, considering that the plant stands obtained by vegetative propagation were genetically, from the point of view of ontogenesis too, of the same age. Since in the case of a perennial *S. dulcamara* the production is influenced not only by current factors but also by effects from the previous year, so from the production results (e. g. weight) of a certain year we cannot directly conclude on the favourable or unfavourable nature of the habitat. The regression coefficients, however, seem to offer a possibility of evaluating and comparing the ecological effects of successive years.

Discussion

In the course of studying the influence of the habitat we found plot B) to display higher production values than plot A) both in 1971 and 1972. The difference between the two plots was, however, essentially smaller in 1972, as shown also by the above characterized regression coefficients, too. In our opinion the difference between the effects of the two habitats can be explained, first of all, by the substantial difference in the amounts of precipitation from the beginning of the vegetation period to the harvest of the plants. In 1972 the extremely large amount of precipitation caused an excess of moisture in the heavy soil of plot B), while on the sandy soil of plot A) exerted a favourable effect. In 1971, on the other hand, the lower amount of precipitation — while providing better moisture conditions in the soil of plot B) — was not sufficient for plot A) owing to the light, sandy character of its soil. The unfavourable effect of the excess soil moisture occurring in plot B) in 1972 corresponded to our earlier observations concerning the flood areas (MÁTHÉ—MÁTHÉ Jr. 1972).

The influence of the habitat on the weight production caused little change in the dry weight ratios of the different plant organs, while the fresh weight values showed obvious differences. On the higher moisture content soil the leaf and stem showed a higher rate of desiccation while with the green fruit it was the other way round. In the latter the ripening stage also has an influence on the rate of desiccation inasmuch as the ripe fruit always contains less water, than the green one, so in the case of the fruit the moisture content is more difficult to bring into direct relation with the ecological effects; it seems to be necessary, however, to study it in connection with the processes of ripening.

When comparing the average alkaloid percentages of the different plant parts in the two habitats we find that in habitats characterized by higher regression coefficient values the leaf and stem show higher alkaloid percentages. The alkaloid content of the fruit is also influenced by the stage of ripening (SANDER 1963a, WILLUHN 1967), so any correlation of this kind is very difficult to establish.

As to the joint action of soil and climatic conditions on the weight as well as percentage and total alkaloid production of *Solanum dulcamara*, our investigations have revealed that on our experimental area the better properties of the soil found on one occasion to be more favourable from the aspect of production do not come into full display when the climatic conditions — first of all the amount of precipitation — are not satisfactory.

To compare the effects of the ecological factors we deem it useful to determine the total shoot-lengths of a number of plants collected from a given site, and plot the weights of leaf, stem, etc. as their function. The regression coefficient of the equation expressing the correlation seems to be suitable for the numerical characterization of the ecological effects in the given year. Inasmuch as the usefulness of these regression coefficients in characterizing the habitat of *S. dulcamara* proves true in further investigations, it would be justified to employ them, as coefficients indicating the productivity of leaf, stem, etc., for the concise characterization of the habitat.

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WHAT IS THE POSSIBLE ROLE OF GIBBERELLIN IN THE BREAKING OF POTATO DORMANCY?

I. PHYSIOLOGICAL EFFECTS OF GA_3 ON CARBOHYDRATE METABOLISM, AMYLASE ACTIVITY AND RESPIRATION IN SPROUTING POTATO

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The possible role of GA_3 in carbohydrate metablism, amylase activity and the promotion of respiration was studied. It was found that the GA_3 treatment around the buds and further, increases the respiration in the storage tissues as compared with control tubers. Parallel to the increase of the intensity of respiration the starch hydrolysing activity of amylase increases too, in the most expressed way around the buds and to a less extent in parts of storage tissues farther from them. All these effects cannot be experienced if simultaneous to GA_3 treatment ABA is introduced, that completely inhibits the generating effect of GA_3. The amount of reducing sugar depends only to a negligible extent on the increase of pH caused by GA_3 treatment, much more upon the GA_3 concentration. Under sterile conditions experiments carried out with tissue slices of potato tubers proved that the effect of GA_3 appears in increasing the "de novo" synthesis of amylase molecules. The amylase synthesis inducing effect of GA_3 can be compensated by ABA.

Introduction

The best known of the physiological effects of the gibberellins are their stimulating effects, such as those breaking dormancy (Brian 1959) or stimulating germination (Szalai—Nagy 1968, Brian—Hemming 1955) but their role in influencing enzymatic transformations is less well known (Hayashy 1940, Paleg 1960). A recurrent idea in the literature is that the enzymatic activity of the endosperms of germinating seeds depends on the enzymes secreted by the embryo and diffusing into the endosperm.

In their experiments on barley grain (Kirsop—Pollack 1958, Pollack 1958) found a positive correlation between the presence of the embryo and the amylase of the endosperm. After the removal of the embryo the amylolytic function of the endosperm was strongly decreased. However, it is not directly the amylase which is secreted from the embryo, but some other substance. This conception was supported by the observations of the Japanese research worker Hayashi (1940), who was to increase the amount of extractable amylase by the addition of exogenous gibberellin (GA_3) to germinating barley grains, without initiating or enhancing the activity of the enzyme at the same time. The conception thus developed that the increase of the concentration of amylase in the endosperm of the germinating barley grain can be attributed to the effect of gibberellin of embryonal origin. Sandegren—Beling (1958)

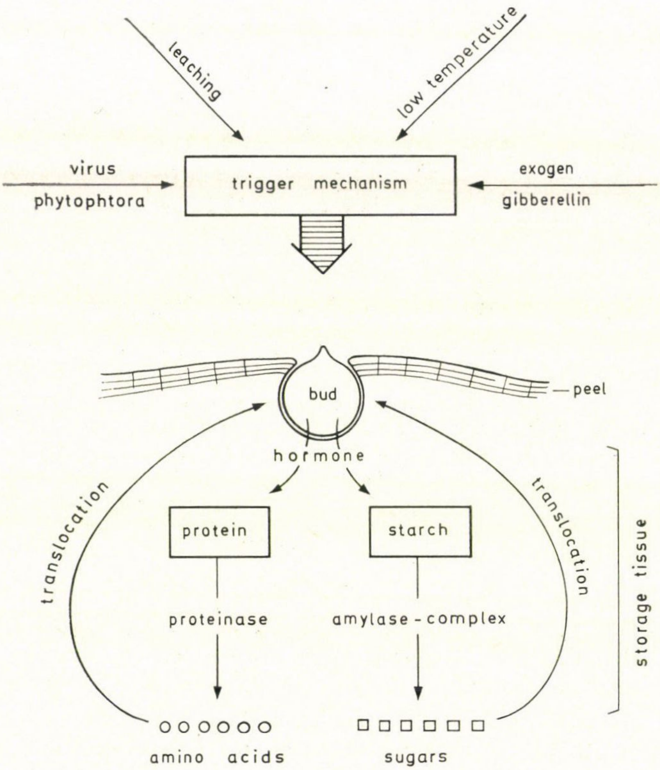

Fig. 1. The assumed effect of exogenous gibberellin on the metabolic changes of potato tubers in a dormant state. Explanation in the text

succeeded in promoting the germination of intact barley by GA_3. According to POLLACK (1958) this gibberellin effect is restricted only to the embryo.

The experimental results mentioned, and many others too. clearly permit the conclusion that, as in germinating barley, endogenous gibberellin may also play an important part in the processes of activation of buds in a state of dormancy. These results further promt the carrying out of investigations to confirm this assumption in the storage tissues of gibberellin-treated potato tubers.

Since it has proved possible in a number of cases to break the dormancy of buds by treatment with exogenous gibberellin, the idea naturally arises that a part might be played in the initiation of the "sprouting" of the potato tuber by the endogenous gibberellins. Setting out from the generally accepted fact that dormant buds, including those of the potato tuber, are extremely poor in growth stimulating substances, while they abound in growth inhibitors, it is assumed that when the potato tuber begins "sprouting", either spontaneously or as a result of some artificial action, the proportions of the regulating substances change. A significant accumulation of gibberellin and auxin can be detected in the surface layers of the tuber, and mainly in the vicinity of the buds and at the same time the amount of inhibitors decreases. This fact was confirmed earlier in the case of tubers stimulated to sprout with rindite.

Our working hypothesis with regard to the study of the hormonal effect of gibberellin is outlined in the following scheme (Fig. 1).

It is assumed that on the addition of exogenous gibberellin metabolic changes begin in the dormant buds, which lead to sprouting. The exogenous gibberellin makes up for the missing gibberellin-synthesizing ability of the dormant buds, and by diffusing into the tissues it initiates the hydrolysis of the stored substances (proteins, starch). The mobilized substances (amino acids, sugars) are then translocated into the buds and begin growing.

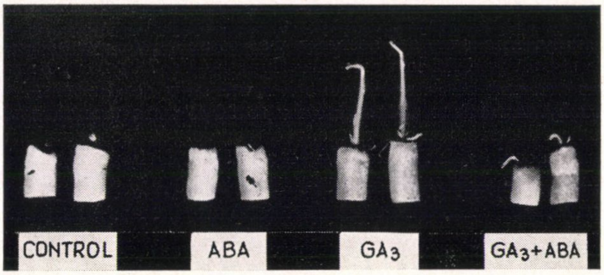

Fig. 2. The reaction of excised dormant buds to GA_3 and ABA. The GA_3 and ABA were introduced on an agar medium acting as substrate. The photo was taken 12 days after setting up the experiment

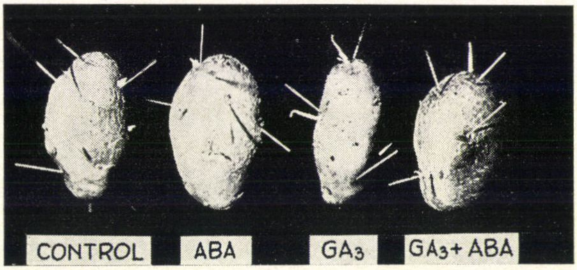

Fig. 3. The effect of GA_3 and ABA introduced into intact tubers via glass capillaries on the sprouting of buds

With the aim of verification our experiments were established on agar media containing GA_3 and ABA; it was observed that the buds germinated rapidly compared with the control due to the action of GA_3, whereas the ABA maintained the dormancy further. On the joint addition of GA_3 and ABA, the growth could be completely repressed, depending on the proportions of the additives (Fig. 2).

This experiment was repeated with intact tubers, when the active agent was introduced via glass capillaries (Fig. 3). Similar results were obtained.

Materials and Methods

Respiration. 5 potato tissue cylinders with buds and 5 without, prepared with a 6 mm diameter cork borer, were incubated at 28 °C in the dark for 20 hr in a sterile Petri dish, on double filter paper moistened with 3 ml sterile water or with 3 ml of a solution containing 200 gamma GA_3. The respiration intensities of the tissue cylinders were determined in a Warburg vessel, in a similar liquid, after 24, 32, 40, 48, 56 and 64 hours. Three parallel series of experiments were carried out. At the end of each experiment the weight of the tissues was measured.

Sugar analyses. 1 gram amounts of tissue cylinders kept under the experimental conditions described above were homogenized in a Potter apparatus after 24, 32, 40, 48, 56 and 64 hours; the volume was made up to 10 ml with bidistilled water and, after the addition of 1 gram Amberlite I, R-120-H ion-exchange resin (frequent shaking for 15 minutes), the mixtures were filtered. The reducing sugar was determined photometrically in a definite amount of the filtrate.

Amylase activity. The amylase activity was established by incubating with the same amount of soluble starch in 0.15 M phosphate buffer (pH 7.0) for 20 minutes at 40 °C. The reducing sugar was determined after the reaction was stopped (SZALAI—FRENYÓ 1962).

Chromatography. Amounts corresponding to 50 μl were transferred to Sch-Sch 2043/b paper from the gibberellin-treated and untreated (control) tissues, equilibrated at 25 °C above a mixture of butanol: acetic acid: water (V/V 4 : 1 : 5), and then chromatographed. The spots were sprayed with aniline-diphenylamine phosphate reagent, and the break-down products were determined.

The GA_3 solution used in the experiments was freshly prepared, and the water was bidistilled. For purposes of sterilization, streptomycin was added to the test solution in all experiments.

Results

The first measurable signs of the effect of the gibberellin are the sudden increase of the intensity of respiration, and the rapid hydrolysis of the starch. Therefore it was necessary to measure the respiration activity of the tubers. O_2 consumption in the different tests was measured in the usual way with a Warburg manometer, and the results obtained were referred to 1 gram dry weight (Fig. 4). When the gibberellin is present, the O_2 consumption increases both in the tissue cylinders containing buds and those without them, while in the absence of gibberellin there is no significant change in respiration intensity.

To state the possible role of bacteria in the intensive increase of respiration, germs were counted from the solutions of a Warburg flasket by agar surface streaking. This test has proved that in the presence of streptomycin during the 64 hour duration of the experiment the medium is practically sterile.

Parallel to the respiration measurements, the amounts of reducing sugar were also determined in these same tissue cylinders, and were expressed in gammas per gram dry weight. Much reducing sugar is formed in the individual tissue samples as a result of the gibberellin treatment (Fig. 5).

To decide whether the sugar formation came from the enzyme effect the experiment was repeated in a way that prior to GA_3 treatment the tissues cut out were put for 30 minutes in hot vapor. The result of the experiment showed that gibberellin exerts an effect upon the enzymes, therefore no sugar formation is experienced in the vapor-treated tissues, i.e. glucose formation is a secondary process (Fig. 6).

The sort and amount of sugar formed was determined by chromatography. Fructose (at Rf 0.49), glucose (Rf 0.44), maltose (Rf 0.3) were identified, and two saccharoselike sugar patches were found (at Rf 0.28 and 0.20), which have not been identified so far.

Although GA_3 is not a strong acid, used in a higher concentration (200 γ/l). To decide whether the pH of the medium affects the formation of the reducing sugars, tissue pieces were incubated in a solution, the pH of which was adjusted by phosphate buffer solution to pH 3.6; 4.7; 5.9 and 7.0 however, in the first three cases there was no hydrolysis. Therefore it was stated that the amount of the reducing sugar depends much more upon the amount of GA_3 present than upon the changes in pH caused by different concentrations. The aqueous control had a pH of 6.9.

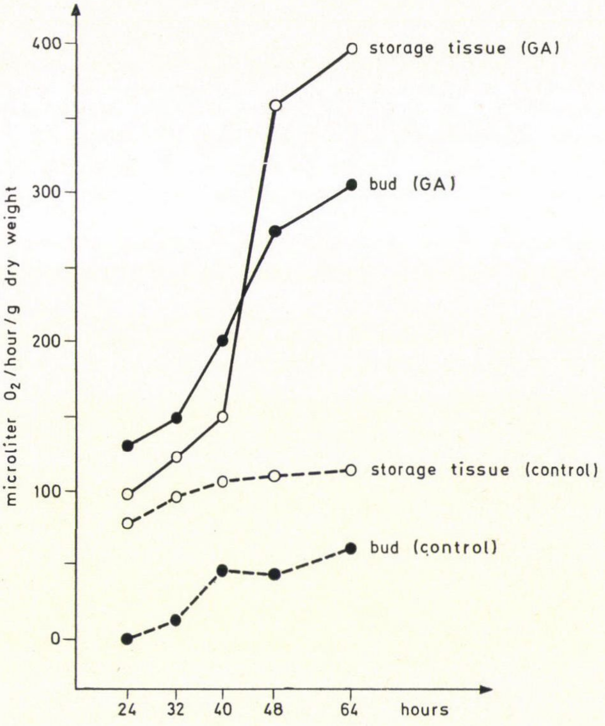

Fig. 4. O_2 consumption in excised potato tissues tested with GA_3 in different ways and for different lengths of time

The effect of gibberellin on the activity of amylase is manifest either in all enzyme molecules otherwise present producing more sugar from the starch (activity stimulation), or in the increase of the number of amylase molecules present. If the gibberellin stimulates the enzyme molecules, then if gibberellin and starch are added to the substrate, the sugar content must increase compared to that of the control without gibberellin. On the other hand, if the effect of gibberellin is based on the increase of the number of amylase molecules (attributable to synthesis *de novo*), then a certain amount of amylase must be found in the solution, since it is water-soluble.

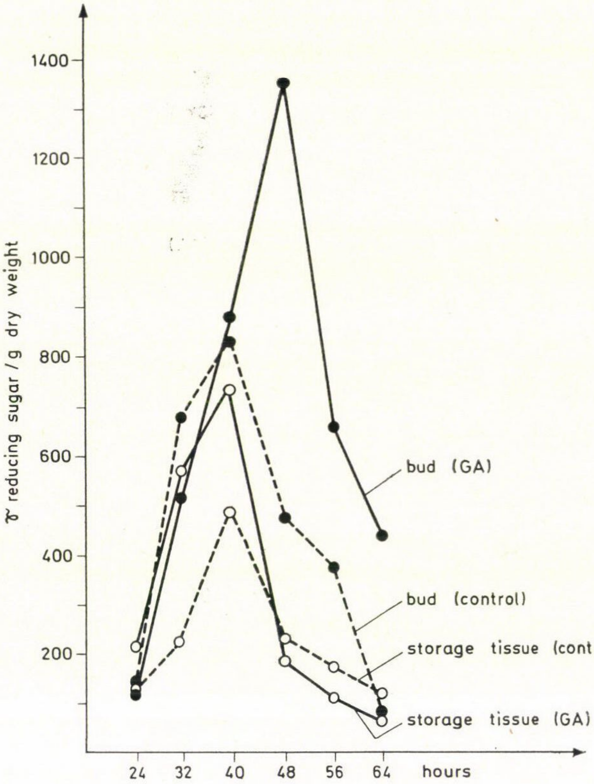

Fig. 5. The changes in the amount of reducing sugars in tissues treated with GA_3 and the untreated controls

It was hoped to obtain an answer to this question from the following experiment.

Tissue discs 10 mm in diameter were placed in Petri dishes, under sterile conditions, in the following three variations:

(1) streptomycin + buffer (pH 6.6) + 3 ml 200 γ/ml GA_3
(2) streptomycin + buffer + 3 ml H_2O (control)
(3) streptomycin + buffer + 3 ml H_2O.

In all three the tissue discs were incubated for 24 hours at 22 °C and then analyzed as follows:

(A) In all three series the amount of sugar diffusing out was determined, the following numerical data being obtained:

γ reducing sugar/g fresh weight

1	2	3
436	375	362

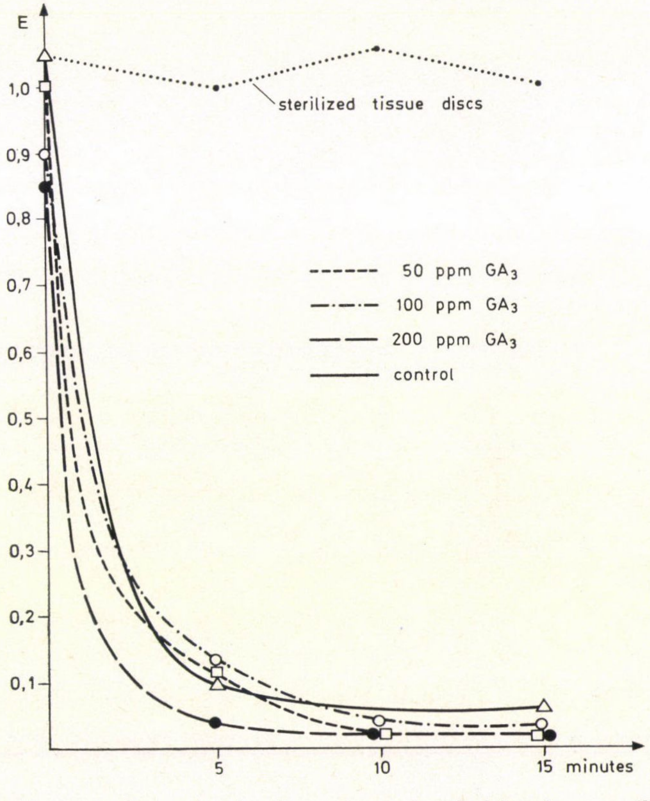

Fig. 6. The reducing sugar formation for tissues treated with GA_3, those sterilized with vapor and the control

(B) All three series were homogenized, the amylase enzyme content was extracted, and with this crude enzyme extract three series of in vitro experiments were carried out:

(a) 1 ml soluble starch + 0.5 ml enzyme preparation (which derive from the 1st series) + 0.5 ml H_2O,

(b) 1 ml soluble starch + 0.5 ml enzyme preparation (which derive from the 2nd series) + 0.5 ml H_2O (control),

(c) 1 ml soluble starch + 0.5 ml enzyme preparation (which derive from the 3rd series) + 0.5 ml 100 γ/ml GA_3.

In all three series "in vitro" experiments were incubated for 20 minutes. The results were as follows:

γ *reducing sugar/g fresh weight*

1	2	3
462	332	368

It can definitely be stated that the sugar excess in experiment (1) may be attributed to the synthesis *de novo* of the enzyme; that is, due to the action of gibberellin the number of enzyme molecules increased. In contrast with this, experiment (3) confirms that the activity of the enzyme molecules present is not increased during a 20 minute incubation with gibberellin.

On the basis of these results, it is assumed that gibberellin (in the absence of the bud) induces an increase in the number of active enzyme molecules in the storage tissue.

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DETERMINATION OF BEHAVIOUR NORMS IN CATTLE OF VARIOUS AGE AND PURPOSE

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The adaptation of cattle of various age and purpose to the applied technologies, or in other words, the extent of co-ordination between the technological systems and the biological demands of animals can be determined in the first place by the behaviour characteristics. The behaviour characteristics (lying, moving, feeding, rumination, water-drinking, defecation, urination) of different age calves and heifers, of cows kept under different conditions, as well as of young fattening bulls of different live weight were studied. The data are characteristic of the examined groups of animals, as on the basis of a mathematical-statistical evaluation the number of observations is sufficient for a generalization. The data reveal that the different keeping methods, if satisfying the demands of the animals, do not modify the course of the major life processes. Under Hungarian conditions of calf rearing, cow keeping and fattening the data are characteristic of the Hungarian spotted breed. As regards the time and frequency of lying and moving, and the number of urinations and defecations the different genotypes showed nearly the same behaviour, so the data established for the Hungarian spotted breed can be extended to other genotypes too.

Introduction

The reaction of a population, and within it of the different groups of animals to a given technology is of decisive importance in keeping farm animals. Namely, to be able to fully exploit the advantages of the industrial character, large-scale animal keeping methods, we have to pay greater attention not only to the directly measurable output, but also to the behaviour of animals indicating their capacity for self-adaptation. The animal reacts to the effects of the environment with different behaviour patterns. The wider the range of either the character or the intensity of these effects, the more difficult the adaptation of the animal. It is obvious that the capacity of the population or the individual for self-adaptation manifests itself in its behaviour as well. The self-adaptation capacity of the population can be registered first of all by the behaviour characteristics. The knowledge of the behaviour data, therefore, offers great help in planning and elaborating the technologies. Namely, the biological demands of the animals cannot be left out of consideration. The interaction of animal and environment must be determined and — as far as possible — made measurable. In this way we shall be able to decide how well we have succeeded in co-ordinating the technological systems with the biological demands of the animals.

On the above consideration we had better establish the behaviour characteristics which are of decisive nature. Each animal has behaviour norms characteristic of the species, and within this behaviour patterns following from its sex and breed. These are — at least according to our present knowledge — conservative. There are, at the same time, behaviour characteristics which can be modified according to the environment. Behaviour patterns accompanied by satisfactory production results can be considered as characteristic of a population. In this case the average behaviour data are assumed to express the typical biological demands. This paper presents some major behaviour data which — while primarily characteristic of the Hungarian spotted breed — can be used in the technological planning for establishing the parameters of other populations too. This offers a possibility of using the data as standards for comparison.

In the international literature behaviour data characteristics of the cattle species and breeds can mostly be found for grazing animals. OLOFSSON (1964), HIMMEL (1965), KOCH (1968) and others studied the different life processes first of all under natural conditions, in the pasture. Observations on stabled animals were generally made with a smaller number of animals, so their validity is limited (BÁRCZY—CZAKÓ 1962, CZAKÓ 1967, REINBRECHT 1968, TSCHIRCH—SOMMER 1970, URBAN 1970, SCHÖN 1971, ANDREAE 1973, and others).

There have been, however, numerous publications on the social patterns of group keeping (BOUISSOU 1965, LIEBENBERG 1965, BRANTAS 1968, SAMBRAUS 1970, and others).

The behaviour trends of calves have also been dealt with by a number of papers (CZAKÓ *et al.* 1966, URBAN 1970, ZEEB—MACK 1971, KITTNER 1969, and others).

On the behaviour of fattening cattle hardly any information is available (DITTING *et al.* 1970).

The effect of different technological systems on the behaviour of cows is also one of the frequently studied factors. The results are rather contradictory (FITZE 1972, WANDER—FRICKE 1967, KOLLER—HAMMER 1972, and others).

The manifold character and high diversity of the observations explain the fact that some authors did not find significant differences between the breeds as regards resting, feeding and rumination (HAUPTMANN 1969), while according to the others there are considerable differences even between closely related breeds (HANCOCK 1954, VAN DER KLEY—VAN DER PLOEG 1955).

Material and Method

Observations required for the establishment of the behaviour norms were made at eight farms on 122 calves of different age, 38 heifers, 76 cows and 223 young fattening bulls with different live weights. All animals belonged to the Hungarian spotted breed.

The observations were made at farms where the production results suggested the satisfaction of the feeding and keeping demands of the animals. At these farms the milk production was 3000—3500 kg, the average daily weight gain 1100—1300 g in fattening and 800—900 g in calf rearing. The calves, heifers and young bulls were kept in groups, while the cows in closed bound, closed free-pen and open free-pen system stables, respectively.

The data were collected in two phases of 48 hours each, partly by continuous observation, partly by recording every ten minutes. A part of the continuous observations on cows bound in pens was made by means of registrating instruments. The "n" values in the tables show the number of animals and not the number of observations. The observation data were evaluated by mathematical-statistical methods, at a 95% confidence level ($P = 5\%$).

Results

The data evaluated on the basis of the observations are presented in Tables 1—15. Table 1 shows the trend of the time spent in lying by 3—24 weeks old calves. When expressing the time of lying as a percentage of a 24 hours period we find that the young, 3—5 weeks old calves spend more time in lying than the older, 14—16 and 22—24 weeks old ones.

The differences are significant. The calves spend more than 50 per cent of the day in lying. Table 2 shows the time spent by the calves in feeding. According to the data — as duly expected on the basis of the differences of feeding and development — the young, 3—5 weeks old calves spend less time in feeding than the 10—12 weeks old or older ones. The differences are significant. Time spent by the calves in rumination — as seen in Table 3 — only takes 11—15 per cent of the day at the age of 3—5 weeks. Later the time spent in rumination is more than 20 per cent.

Table 1

Time spent in lying within 24 hours by calves of different age kept in groups (n = 116)

Age of the calves	Average minutes $\bar{x}$	Standard deviation ± s	Average percentage of 24 hours	Extreme values as a percentage of 24 hours	
				max.	min.
3—5 weeks	957.4	113.6	66.4	75.09	50.80
10—12 weeks	849.6	84.2	59.0	68.30	76.33
14—16 weeks	793.4	102.1	55.1	57.80	43.20
22—24 weeks	768.9	96	53.4	63.70	43.80

3—5 weeks old calves > 14—16 weeks old ones, P = 5%
3—5 weeks old calves > 22—24 weeks old ones, P = 5%

The occurrence of lying in 24 hours (Table 4) does not change with the advancing age of calves, while at the same time the frequency of motion in-

Table 2

Time spent in feeding during 24 hours by calves of different age kept in groups ($n = 212$)

Age of the calves	Average minutes $\bar{x}$	Standard deviation ± s	Extreme values as percentage of 24 hours		Average percentage of 24 hours
			max.	min.	
3—5 weeks	133.9	32.5	15.0	5.8	9.3
10—12 weeks	257.4	51.2	22.3	7.2	17.9
14—16 weeks	260.6	49.7	23.9	8.8	18.1
22—24 weeks	321.1	52.1	30.20	14.47	22.3

3—5 weeks old calves > 10—12 weeks old ones, P = 5%
3—5 weeks old calves < 14—16 weeks old ones, P = 5%
3—5 weeks old calves < 22—24 weeks old ones, P = 5%

Table 3

Time spent in ruminating during 24 hours by calves of different age kept in groups ($n = 108$)

Age of the calves	Average minutes $\bar{x}$	Standard deviation ± s	Extreme values as a percentage of 24 hours		Average percentage of 24 hours
			max.	min.	
3—5 weeks	190.0	29.2	18.8	7.9	13.2
10—12 weeks	300.9	57.1	27.9	13.5	20.9
14—16 weeks	324.0	40.6	35.4	15.8	22.5
22—24 weeks	332.6	49.8	39.7	17.0	23.1

3—5 weeks old calves < 14—16 weeks old ones, P = 5%
3—5 weeks old calves < 22—24 weeks old ones, P = 5%

creases. According to the data of Tables 5 and 6 the daily occurrences of feeding, rumination, defecation and urination are almost the same from 3—5 to 22—24 weeks of age. The calves feed and ruminate on 9—14 occasions a day, defecation occurs on 9—11, urination on 4—5 occasions.

Table 7 shows the time spent by 10—12 and 18—21 months old heifers in lying, feeding and rumination a day. According to the data of the table the heifers spend 46—48 per cent of the day's 24 hours in lying, 16—18 per cent in feeding and 20—22 per cent in rumination. Age does not cause significant differences in the behaviour patterns. Table 8 gives information about the occurrences of lying, ruminating, water-drinking, as well as on the frequency of defecation and urination.

Table 4

Frequency of lying and moving during 24 hours with different age calves kept in groups (n = 116)

Age of the calves	Occurrences of lying			Occurrences of moving		
	average	extreme values		average	extreme values	
		max.	min.		max.	min.
3—5 weeks	16.2	24	8	18.6	27	10
10—12 weeks	15.4	21	7	19.2	30	16
14—16 weeks	13.2	19	6	29.7	28	15
22—24 weeks	14.8	21	6	28.2	41	16

Table 5

Frequency of feeding and ruminating during 24 hours with different age calves kept in groups (n = 122)

Age of the calves	Occurrences of feeding			Occurrences of ruminating		
	average	extreme values		average	extreme values	
		max.	min.		max.	min.
3—5 weeks	9.6	16	5	10.6	13	5
10—12 weeks	11.2	20	7	12.7	18	9
14—16 weeks	13.5	17	8	13.8	21	9
22—24 weeks	14.3	19	8	14.2	22	10

Table 6

Frequency of defecation and urination during 24 hours with different age calves kept in groups (n = 52)

Age of the calves	Occurences of defecation			Occurrences of urination		
	average	extreme values		average	extreme values	
		max.	min.		max.	min.
3—5 weeks	10.2	17	6	4.2	9	3
10—12 weeks	11.1	18	7	4.8	10	3
14—16 weeks	9.2	20	7	5.3	10	3
22—24 weeks	10.7	17	6	5.1	11	4

Rumination is more frequent with the heifers than feeding. Table 9 shows the time spent in lying and moving by cows kept in different ways. According to the data of the table the cows spend 42—47 per cent of the day's

Table 7

Time spent in lying, feeding and ruminating by heifers kept in groups (n = 38)

Life processes	Age of the heifers 10–12 months	Age of the heifers 18–21 months
Lying		
average minutes, $\bar{x}$	666.7	694.0
standard deviation, ±s	132.3	147.2
average % of 24 hours	46.3	48.2
max. % extreme values	65.7	70.9
min. values	35.6	40.4
Feeding		
average minutes, $\bar{x}$	239.0	260.6
standard deviation, ± s	62.4	57.8
average % of 24 hours	16.6	18.1
max. % extreme values	27.9	29.2
min. values	11.7	13.4
Rumination		
average minutes, $\bar{x}$	292.3	318.2
standard deviation, ± s	62.5	56.4
average % of 24 hours	20.4	22.1
max. extreme values in %	32.03	30.7
min. extreme values in %	12.7	13.5

24 hours in lying. As regards the time of lying there is no significant difference between the bound- and the free-pen system of keeping.

The differences are not significant. In the case of free-pen keeping the time spent in walking is very little, not more than 1.2—1.4 per cent. Table 10 shows the data of feeding and rumination. In a system based on mass fodders and dairy supplements the cows spend 15—18 per cent of the day in feeding, and 24—27 per cent in ruminating. As regards feeding and rumination the differences between the keeping systems are significant. Table 11 presents the number of the lying periods as well as the occurrences of defecation and urination. The cows generally lie down on 6—8 occasions a day. In the free-pen keeping system the lying periods of cows are more in number than in bound keeping. The differences are significant. Defecation occurs on ten, urination five-six occasions, on an average.

Table 8

Daily frequency of life processes with heifers kept in groups (n = 38)

Frequency of life processes in 24 hours	Age of heifers	
	10–12	18–21
	months	
Occurrences of lying		
average	8.2	8.6
max.	17	16
min.	5	6
Occurrences of rumination		
average	10.3	11.4
max.	17	18
min.	5	6
Occurrences of water-drinking		
average	6.2	6.8
max.	14	16
min.	4	3
Occurrences of defecation		
average	9.2	9.8
max.	14	13
min.	5	5
Occurrences of urination		
average	6.2	6.4
max.	10	11
min.	3	4

As to the frequency of feeding there are differences between the cows kept in bound-system and free-pen system stables, respectively (Table 12), in spite of the fact, that the feed was supplied in rations in both cases. In the free-pen system the cows consume their rations in more steps than in the bound system. The differences are significant. Differences of this kind are not found in the frequency of water-drinking and ruminating.

Times spent in lying and moving by young fattening bulls of different live weight — i.e. examined in different phases of fattening — are presented in Table 13. According to the data of the table there is no considerable difference between the young fattening bulls of different live weight as regards the

Table 9

Time spent in lying and moving during 24 hours by cows kept under different conditions

Designation	Keeping system		
	closed bound $n = 36$	closed free $n = 24$	open free $n = 16$
Lying			
Average minutes, $\bar{x}$	681.1	616.3	650.8
standard deviation, $\pm$ s	101.3	117.8	118.4
average % of 24 hours	47.3	42.8	45.2
extreme values in %			
max.	65.3	63.2	64.4
min.	22.9	20.2	18.8
Motion			
average minutes, $\bar{x}$	—	17.2	20.1
standard deviation, $\pm$ s	—	9.2	8.5
average % of 24 hours	—	1.2	1.4
extreme values in %			
max.	—	5.4	5.6
min.	—	0.5	0.6

time spent in lying; when kept in groups they spend 54—58 per cent of the day's 24 hours in lying. The differences between the weight-groups are not significant.

In the weight category of 300—400 kg the time spent in moving is essentially less than in the weight group of 200—300 kg.

Owing to the relatively high standard deviation significant differences are only found between the 200—300 kg group and the animals with live weights over 400 kg. Table 14 contains again the times spent in feeding and ruminating. When dry mixtures are fed, the different live-weight young fattening bulls spend 7—9 per cent of the day in feeding and 17—22 per cent in ruminating. Differences between the averages of the individual groups are not significant. Table 15 shows the frequency of feeding, ruminating, water-drinking, defecation and urination in a 24 hour period of observation. According to the data of the table young fattening bulls of different live weight feed on 5—6 occasions and ruminate in 8—9 phases a day. The occasions of lying are 7—8, those of water-drinking 5—6 in number. The occurrences of defecation are 5—7, those of urination 4—5 a day, on an average.

Table 10

Time spent in feeding and ruminating during 24 hours by cows kept under different conditions

Designation	Keeping system		
	closed bound $n = 36$	closed free $n = 24$	open free $n = 16$
Feeding			
average minutes, $\bar{x}$	261.1	218.8	240.9
standard deviation, $\pm$ s	44.2	52.6	38.7
average % of 24 hours	18.2	15.2	16.7
extreme values in %			
max.	25.8	29.6	34.4
min.	3.8	5.77	4.8
Rumination			
average minutes, $\bar{x}$	391.3	346.0	342.7
standard deviation, $\pm$ s	47.3	62.1	54.6
average % of 24 hours	27.3	24.1	23.8
extreme values in %			
max.	35.9	38.97	38.1
min.	23.48	20.9	18.0

Discussion

The observation results were first evaluated from the point of view of whether the obtained data were characteristic of the studied groups of animals and suitable for establishing their norms of behaviour. Therefore the approximate number of data required for a possible generalization was already decided on when planning the experiment. In estimating the necessary number of observation data the following statistical test was used to make sure of the correctness of the hypothesis:

$$n = \frac{tp\%^2 \cdot s\%^2}{h\%^2}$$

n = number of observation data required to obtain a reliable result,
$tp\%$ = theoretically calculated (Student's trial statistics) critical value at $P = 5\%$ probability level, and infinite liberty,
$s\%$ = standard deviation given in the form of a variation coefficient,
$h\%$ = chosen percentage of estimation error.

Table 11

Frequency of lying, defecation and urination during 24 hours with cows kept under different conditions

Frequency values	Keeping system		
	closed bound $n = 36$	closed free $n = 24$	open free $n = 16$
Lying			
average	6.9	8.2	7.2
max.	16	17	14
min.	4	5	3
Defecation			
average	10.3	9.6	10.1
max.	18	17	18
min.	4	6	5
Urination			
average	6.4	5.6	5.5
max.	10	9	8
min.	4	5	4

Lying: closed bound < closed free, P = 5%

The value of $p\%$ was determined at $P = 5\%$ from the table of t-values. The permissible error of estimation was taken as 3 per cent.

In the experiments carried out so far the highest variation was found in feeding and rumination. The highest variation coefficient was 28 per cent. The possibility of error was determined at $\pm 3\%$. In this case the number of necessary observations is

$$n = \frac{(1.9)^2 \cdot (28)^2}{3^2} = 314$$

that is, 314 data are required. With the twice 48 hours observation the number of samples taken even in the group with the lowest number of animals was higher than what had been determined in advance as a minimum. Since the assumed 28% variation coefficient was only approximated by the standard devation values of feeding rumination, the reliability of the data in this respect was further increased. Thus, on the ground of the obtained results the values presented in Tables 1—15 can be accepted as characteristic of the behaviour of Hungarian spotted cattle of the given age and purpose.

Table 12

Frequency of feeding, water-drinking and ruminating during 24 hours with cows kept under different conditions

Frequency values	Keeping system		
	closed bound $n = 36$	closed free $n = 24$	open free $n = 16$
Feeding			
average	10.2	13 6	13.2
max.	8	7	8
min.	14	16	17
Water-drinking			
average	5.6	4.8	5.1
max.	3	3	4
min.	8	6	7
Rumination			
average	12.6	9.8	10.1
max.	9	8	7
min.	17	18	17

Feeding: closed bound < closed free and open free; P = 5%

From the data of the tables it is further seen that though the extreme values — concerning both the upper and lower limits — are rather high, the standard deviation shows a relative value of 10—20 per cent. So most of the obtained parameters do not deviate considerably from the mean value.

Of the individual characteristics of behaviour it is in the case of lying, feeding and rumination that the differences due to the age of calves are significant. On the other hand, the differences found between the behaviour data of one-year old heifers and those of one and a half year old ones are not significant. Differences shown in the mean values of the behaviour data of cows kept in bound- and free-pen system stables, respectively, are not significant. Thus, if the system of keeping satisfies the demands of the animals, it does not considerably influence the course of the major life processes (lying, feeding, rumination, water-drinking, urination and defecation).

As to the daily periods of lying, feeding and rumination there was no considerable difference between the studied live weight categories of young fattening bulls. The differences between the weight groups are not significant. It was only in the average value of motion that the 200—300 kg live-weight

Table 13

Time spent in lying and moving by different live-weight young fattening bulls kept in groups under intensive fattening conditions based on dry mixture

Designation	Live-weight		
	200—300 kg $n = 68$	300—400 kg $n = 72$	over 400 kg $n = 83$
Lying			
average minutes, $\bar{x}$	832.4	790 9	799.8
standard deviation, $\pm$ s	70.4	61.2	56.7
average % of 24 hours	57.8	54.9	55.5
extreme values in %,			
max.	64.8	57.8	59.1
min.	49.9	49.4	44.8
Motion			
average minutes, $\bar{x}$	24.3	18.2	14.3
standard deviation, $\pm$ s	4.6	3.7	3.1
average % of 24 hours	1.7	1.2	0.9
extreme values in %,			
max.	5.9	3.9	4.4
min.	0.8	0.7	0.5

Motion: 200—300 kg > over 400 kg, $P = 5\%$

group differed to a greater extent from that above 400 kg. Fattening bulls with more than 400 kg live-weight moved less than the younger and lighter ones.

Beyond accepting the results of investigations as characteristic of the Hungarian spotted breed under the calf rearing, cow keeping and fattening condition of Hungary, a comparison with other breeds, types and keeping systems seemed to be useful. According to the author's investigations into the behaviour of the calves the 14—24 weeks old calves spend 768—793 minutes in lying daily. Koch (1968) found the average lying time of calves of the Maine — Anjou breed to be 740 minutes a day. According to Konrad (1967) the average time of lying is 850 minutes with 6—10 weeks old Czech spotted calves. In the present investigations the average lying time of Hungarian spotted calves of about the same age was found to be 849.6 minutes (Table 1).

The average lying time of the black-spotted cows was found by Koch (1968) to be 711 minutes in bound keeping system stables, and 590 minutes in open, free-pen system stables. According to the investigations of Rein-

Table 14

Time spent in feeding and ruminating by different live-weight young fattening bulls kept in groups under intensive fattening conditions based on dry mixture

Designation	Live-weight		
	200–300 kg $n = 68$	300–400 kg $n = 72$	over 400 kg $n = 83$
Feeding			
average minutes, $\bar{x}$	129.8	102.9	102.1
standard deviation, $\pm$ s	22.4	16.8	19.2
average % of 24 hours	9	7.1	7.1
extreme values in %,			
max.	12.20	9	13.0
min.	8.3	6.5	6.1
Rumination			
average minutes, $\bar{x}$	260.8	258.0	306.2
standard deviation, $\pm$ s	50.4	38.6	45.9
average % of 24 hours	18.1	17.9	21.2
extreme values in %,			
max.	23.51	19.7	23.5
min.	12.4	11.8	73.6

brecht (1968) the average lying time of the German black-spotted cows was 639 minutes in bound system stables, and 598 minutes in closed free-pen system stables. The time of rumination was found to be 350 minutes in closed bound system stables. In the experiments carried out by Hauptmann (1969) the average daily lying time of the Canadian Holstein Frisian and Danish red cow (in stables with resting boxes) ranged between 603 and 662 minutes. The high yielding dairy cows spent substantially more time in lying than those producing less milk. The above listed data are nearly identical with average values calculated from the author's observations. The results of investigations carried out by Kovalcik—Chobot (1971) on the time of lying and feeding, and number of defecations and urinations with Slovakian spotted, Danish red and black-spotted cows practically agree with both the listed literary references and the results of the author's own investigations.

The data published on the black-spotted breed practically correspond to the results of the author's investigations. Differences in the time of feeding and rumination are due to the different system of feeding (Ditting *et al.* 1970, Andreae—Thiedemann 1972).

Table 15

Frequency of occurrences of feeding, rumination, water-drinking, defecation an urination with different live-weight young fattening bulls kept in groups under intensive fattening conditions based on dry mixture

Frequency values	Live weight		
	200 – 300 kg $n = 31$	300 – 400 kg	over 400 kg $n = 28$
Feeding			
average	6.2	5.8	6.3
max.	9	11	10
min.	3	3	3
Rumination			
average	9.6	8.7	8.1
max.	13	14	12
min.	6	5	5
Lying			
average	7.8	7.1	7.3
max.	15	14	16
min.	6	6	5
Water-drinking			
average	6.20	5.7	5.1
max.	11	12	9
min.	3	3	4
Defecation			
average	7.7	6.4	5.5
max.	13.0	12.0	14.0
min.	4.0	5.0	4.0
Urination			
average	5.4	5.1	4.1
max.	11.0	8.0	8.0
min.	3.0	4.0	3.0

When comparing the results of the present investigations with the literary data we find that as regards the time of lying and moving as well as the frequency of urination and defecation the different genotypes show nearly the same behaviour. At the same time the periods of feed consumption and rumination depend primarily on the system of feeding. The system of feeding thus has a greater influence on these behaviour characteristics than the genotypes have. For example, in the case of fattening mainly by giving mass fodders the feed consumption takes 15—18 per cent, while by feeding dry mixtures it takes 7—9 per cent of the day.

Thus, in planning the keeping system and technology the behaviour patterns can be applied to other genotypes too, with the exception of the behaviour parameters of feed consumption and rumination.

The processed data of observations thus offer help in elaborating the keeping system (technologies) by giving information on the biological demands of the animals.

Neglect of the biological demands of animals — since the technological systems have been elaborated mainly on the basis of economic considerations — has often been responsible for the unsatisfactory production results.

Beyond giving help in planning the technologies of farms these data — as the behaviour parameters of cattle of given age and purpose — can be used for comparing the capacity for self-adaptation in different populations.

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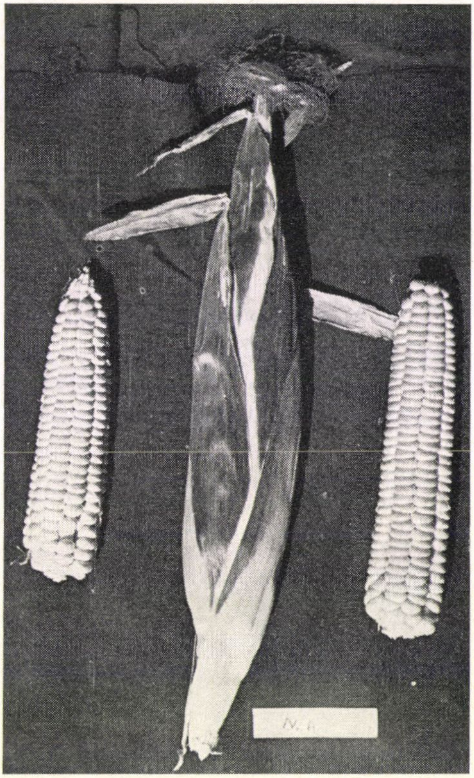
VARIA



"NAGYKÁLLÓI ARANYMAZSOLA" MAIZE

Taxonomical place: *Zea mays* L. convar *saccharata* Koern. cv. *flavodulcis* Koern.
Origin: individual selection from populations of Aranymazsola (Golden Bantam).
Beginning of breeding: 1949, Nagykálló.
Breeders: János Székács and Ambrus Szabó (Nagykálló).
State qualification: provisionally certified variety, 1957.
General characterization: early maturing, healthy, hardy, highly productive, yellow raisin-type sweet corn; suitable for both marketing and deep freezing (Kapás *et al.*, 1965).,

Morphological description:

Root-system: its fibrous root-system penetrates 140—150 cm deep into the soil; tends to form suckers.

Shoot system: consists of a 140—190 cm long main shoot and about two 150 cm long laterals; tillering of medium extent.

Stem: solid, with about 11 internodes of some 2 cm thickness; dark green with red stripes, the nodes are yellowish green and bare.

Foliage: the length of the fifth leaf blade is 75—80 cm, its width about 9 cm; the number of leaves usually is 7—11. The leaf is dark green, but along the main rib usually mottled with red. The leaf sheath is medium hairy, the leaf blade thinly haired.

Inflorescence: the male inflorescence 40—62 cm long with 17—30 laterals on it; the average number of ears is 1.6. The lower inflorescence develops at a height of 45—49 cm above-ground. Ears develop on the laterals too (0.8/plant).

Flowers: anthers and pistil are of reddish green colour.

Ear: length 14—20 cm, diameter an average of 3.8 cm, cylindrical shape; weight 7—11 dkg. The number of grain rows is 10—12, and the number of grains per ear 260 on an average. The cob is white.

Caryopse: when ready for sale light yellow, at the stage of biological maturity golden yellow. The grains are of typical sweet corn shape (raisin-type). Thousand-grain-weight generally is 248 g.

Biological characters:

Vegetation period: generally 123 days of which the vegetative development takes some 50 days and the generative development 73 days. It is ready for sale 67—77 days after emergence. It is generally of early maturation (MÁNDY—KARKOVSZKY 1959).

Water requirement: medium.

Resistance to diseases: fairly resistant, hardy variety (KAPÁS *et al.* 1965).

Farm technology requirement:

Sowing: in the first half of May.

Soil requirement: best grown on medium heavy loam.

Productivity: high yielding variety of very good taste.

Region of cultivation: the entire area of Hungary.

*

Prepared at the Department of Botany, University of Agricultural Sciences, Debrecen.

GY. MÁNDY

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METHODOLOGICAL QUESTIONS OF PIG HYBRIDIZATION

Outstanding results in livestock breeding by the method of hybridization were first attained in the poultry branch. As it is generally known, in pig species, research on hybrid production began, during the last decade. For a long time breeders were of the opinion that in this species owing to the specific characteristics of pigs results similar to those obtained in poultry hybridization could not be attained. This opinion was mainly due to the fact that the pigs showed high reactivity to inbreeding manifested first of all in properties related with fitness. It thus became questionable whether the costs of inbreeding would be proportionate to the expected gain.

A survey is given below of the basic methodological questions of the ten years breeding work carried on by the author at the Kaposvár Agricultural College, and of the results which led to the KA-HYB hybrid pig.

As to the practical results of this breeding work, KA-HYB pigs are kept today on some 200 large farms in Hungary, and in 1974 about 1 million of them will be delivered to the slaughter-houses of the country. The interest shown abroad is also quite considerable; orders are arriving not only from the surrounding countries but from countries of other continents, too. With a view to the organized marketing of KA-HYB pigs a joint undertaking of farms engaged in the production of the breeding material was established in 1968.

I.

Special methods of line breeding

The work of pig hybridization was based — like the hybridization of poultry — on line breeding. Prior to discussing the methods of breeding it has to be noted that in the case of paternal lines it is considered to be of primary importance to develop populations whose breeding is directed to the reasonably attainable homozygote state. In the course of the present work such lines have generally been based on individuals that have proved outstanding in their breed, except one line in which progenies originating from a remarkably good mating formed this basis owing to their additive character values.

1. Methodology of producing paternal lines: In developing the hybrid paternal lines three methodologically different methods of breeding were applied:
   A) Production of pure-bred animals by inbreeding;
   B) Production of synthetic lines;
   C) Development of F_1 combinations.

A) *Production of pure-bred animals by inbreeding*

In line breeding the main point is to develop the gene frequency in the required direction. Inbreeding is given an important role in line breeding. According to the author's experiences the genotypic conformity of a population is more readily ensured by inbreeding. This is the basis of reproducibility which plays an important role in hybridization, and which is much more easy to attain in such a case than in the case of breed populations of a much wider genetic variance. Blood typing that has been carried on for six years is also of great service. It helps in disclosing the genotypic differences between the lines (Table 1) which are taken into account when mating within the line. Peculiar differences existing between the lines can thereby be deliberately increased, and so the lines genotypically differentiated. In this way relatively distant genotypes can be combined in the course of crossing, promising more expressed crossing effects. Blood-typing — as an important aid — is fundamentally relied upon when realizing the above.

Inbreeding when applied in the course of line breeding is flexibly adjusted to the individual tolerance of the animals. Namely, the tolerance of the animals is not uniform but individually depends on the extent of the homozygous character accidentally brought about at the loci of their non-additive genes in the course of the meiosis preceding their birth.

In certain cases immigration is employed in order to maintain the viability of the line. In this case we do not stick to a given breed unconditionally. In the case of animals chosen for the purpose of immigration the most important requirement is that as far as possible in properties transmitted on an additive basis they should be superior to the members of the line they are to be crossed with. This is necessary because such properties are transmitted in an intermediary way. In the particular selection methodology of hybridization — as will be pointed out later — this is of considerable importance. In the present case the main point of immigration is not to introduce additive genes of high value but to neutralize the depression caused by inbreeding at least to such an extent that the reduced fertility should not jeopardize the production of subsequent generations within the line. All this should be done in such a way that the heterozygous character increasing in the non-additive gene pairs should not alter the phenotype of the line nor its characteristics shown in transmittance.

Table I

Differences in blood groups between the lines

Factors	Line CX		Line LIII		Line LI	
A	80.00*	9.36**	34.80*	3.50**	42.10*	4.60**
Bb	5.00	0.58	7.60	0.76	—	—
Ea	12.50	1.40	36.40	3.65	10.50	1.15
Eb	7.50	0.88	62.10	6.24	31.60	3.45
Ed	100.00	11.70	100.00	10.05	100.00	10.92
Ee	95.00	11.11	87.90	8.83	94.70	10.34
Ef	97.50	11.40	18.20	1.82	63.20	6.90
Eg	45.00	5.26	100.00	10.05	52.60	5.75
El	12.50	1.46	36.40	3.65	—	—
Ja	100.00	11.70	81.80	8.22	84.20	9.19
Kb	52.50	6.14	53.00	5.33	42.10	4.60
Kc	55.00	6.43	69.70	7.00	36.80	4.03
Lc	92.50	9.69	81.80	8.22	79.00	8.62
Lg	—	—	78.80	7.91	94.70	10.34
Hp_8	—	—	28.80	2.89	84.20	9.19
Hp_{13}	—	—	18.20	1.83	—	—
Tf AA	2.50	0.30	—	—	—	—
Tf BB	97.50	11.40	100.00	10.05	100.00	10.92

* = percentage frequency of factors
** = percentage frequency of occurrence of factors

Table 2

Trend of maternal performance in the continuant hybridization of KA—HYB

Number of line	Designation	Number of farrows	Number of born piglets	Number of piglets at the age of 21 days	Total weight at 21 days	Percentage loss	Average number born	Average number at 21 days	Average weight at 21 days
1.									
I.		38	385	257	1358	33.25	10.13	6.76	5.28
II./A		960	8534	7936	50248	7.01	8.88	8.26	6.33
II./B		19	166	151	725	9.04	8.74	7.94	4.80
IV.		49	383	342	1770	10.70	7.82	6.98	5.18
VII.		48	470	373	1960	20.64	9.79	7.77	5.25
XI.		119	1050	608	3223	42.10	8.82	5.10	5.30
XLIX.		7	50	44	214	12.00	7.14	6.28	4.86
LI.		10	74	53	284	28.38	7.40	5.30	5.36
CX.		240	2072	1635	8909	20.22	8.63	6.88	5.39
		1490	13184	11417	68691	13.41	8.85	7.66	6.02
2.	Nádudvar	1097	10652	9650	51170	9.41	9.71	8.79	5.30
	Nagyecsed	42	418	382	—	8.62	9.95	9.09	—
	Pincehely	179	1789	1482	8624	17.16	9.99	8.27	5.82
	Várong	112	1021	892	4704	12.24	9.12	8.00	5.25
		1430	13880	12410	64498	10.60	9.70	8.67	5.35
3.	Nádudvar	154	1441	1266	6485	12.14	9.36	8.22	5.12
	Pincehely	468	4490	4227	22892	5.86	9.59	9.08	5.42
	Törökszentmiklós	76	710	622	—	12.39	9.34	8.18	—
	Veszprém	462	4996	4385	22235	12.23	10.81	9.49	5.07
	Nagyecsed	930	8519	7601	—	10.78	9.16	8.17	—
	Agárd	1308	12233	10735	60116	12.25	9.35	8.21	5.60
		3398	32389	28836	111728	10.97	9.67	8.44	5.42

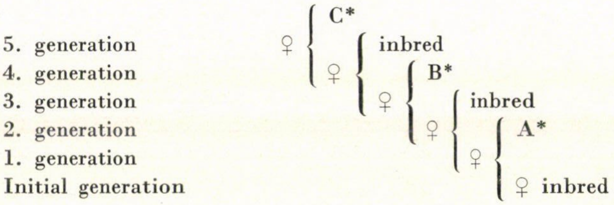
1. = 1972. farrowing data of inbred lines
2. = 1972. farrowing data of sows originating from the first combination
3. = 1972. farrowing data of sows originating from multiple combinations

The data were collected from controlled breeding stocks in which epizootic diseases affecting the production did not occur in the course of the year

The farrowing data include the data of sows producing but a small number of piglets too, further, the 21 days averages are related to the number of sows having farrowed

Some 60 per cent of the production results summed up in point 3, and about 20 per cent of the others are first-gravid performances

It has the following scheme:



This is the schematic solution of immigration in line breeding. When the inbred level of the population attains a marginal depression immigration is applied with the individual marked "A". Male individuals originating from this combination will be sorted out, while the female progenies will be mated with the male parents showing the highest inbred level in the line at that time. Though from the point of view of the line the future progenies are "R_1"-s, since their father and maternal grandmother are highly inbred with the same ancestor, they are — according to experience — full representatives of their line either in phenotype or in transmitting ability. Such female members of the line will then be mated first with the less inbred males of the line in order to find out whether or not the future progeny is sufficiently viable to produce the subsequent generation. If so, this mating is maintained for this generation, and only the female progeny will be mated with immigrated males. If, however, in the course of mating within the line piglets showing a great depression are born, this indicates that this generation can only be reproduced through immigration. It is in such cases that the individual marked "B" is introduced in the population. This system is continued further on.

In this work a flexible inbreeding adjusted to the individual is indispensable, as for the time being it cannot be adjusted to a scheme designed for more than one generation. As another essential requirement, the successive immigrants should by no means be related to one another, in some cases they must not even belong to the same breed. This is the basic precondition of maintaining the dominance of the initial line which is ensured by a regular back-crossing. The experiences obtained in various lines cover 5—7 generations. On this basis, of the methods of line breeding this procedure is considered to be the most feasible way of pig hybridization.

Using this method lines with superfluously large numbers need not be maintained, namely such lines always threaten with the absence of genotypic conformity. On the other hand, a more intensive application of inbreeding-without causing substantial economic losses thus becomes possible. Namely, in the case of lines used as great-grand parents and grand parents only a few progenies are required a year. In this case the maintenance of large populations would only be possible at the expense of either profitability or genotypic conformity. Inbreeding can thus be applied in a more intensive form. This helps in reducing the additive and non-additive genetic variance to the required extent. Immigration made use of from time to time does not loosen the hereditary basis of the population to such an extent as it would do if inbreeding were not repeated.

Thus the essence of the method is the flexible application of a close inbreeding.

When further discussing the question of inbreeding three points may be of main interest:

a) Is a greater effect ensured by crossing inbred partners than when crossing non-inbred ones? It is a world -wide opinion that the inbreeding depression and the heterosis effect are two interrelated counterpoles of the same genetic phenomenon. This would, in fact, favour the application of inbreeding as far as a more significant effect is to be attained. Yet the ques-

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* C, B, A are individuals included in the line through immigration.

Table 3

Losses of sows in the continuant hybridization of KA—HYB

Number of line	Designation	Number of sows	Perished	Emergency slaughtered	Did not rut	Sterile	Total	
							number	%
1. I.		32	4	—	—	6	10	31.3
II./A		771	21	103	37	32	193	25.0
II./B		24	1	1	—	—	2	8.3
IV.		50	6	1	—	—	7	14.0
VII.		19	2	—	—	—	4	21.0
XI.		81	4	2	3	2	11	13.6
XLIX.		25	1	1	—	5	7	28.0
LI.		13	4	—	—	1	5	38.5
CX.		179	16	—	—	24	40	22.3
		1194	59	108	40	72	279	23.4
2.	Nádudvar	556	27	25	7	8	67	12.1
	Nagyecsed	42	4	2	2	1	9	21.4
	Pincehely	89	3	4	1	3	11	12.4
	Várong	99	3	11	—	—	14	14.1
		786	37	42	10	12	101	12.8
3.	Nádudvar	157	4	3	1	1	9	5.7
	Pincehely	324	5	16	1	16	38	11.7
	Törökszentmiklós	60	4	2	2	—	8	13.3
	Veszprém	357	3	38	6	18	65	18.2
	Nagyecsed	562	61	23	20	21	125	22.24
	Agárd	945	11	13	5	14	43	4.6
		2405	88	95	35	70	288	11.9

Notes: 1. = inbred lines
2. = sows from the first combination
3. = sows from the multiple combination

tion cannot unanimously be answered in the affirmative. It is only so in the case when the gene loci of the inbred parents to be mated contain those genes which are able to induce heterosis — intra- and interallele gene-interactions — in the combination. If these genes are absent at the gene loci of the partners, then the lines have been homogenized by inbreeding for nothing,

Table 4

Pooled relative economic index

1971—1972

(On the basis of data of sows having farrowed several times)

Property studied		Large white Landrace F_1	KA–HYB 32	Difference
A. *Breeding results:*				
Number of piglets farrowed by 100 sows	n	954	1012	58
Number of piglets weaned by 100 sows	n	816	873	57
B. *Fattening results:*				
Age at the beginning of fattening (days)		86	86	
Progeny slaughtered per 100 sows	(n)	751	803	52
Daily weight gain while fattened	(g)	653	694	41
Daily weight gain in the whole life	(g)	523	534	11
Strach value used for 1 kg weight gain (operative)	(kg)	2.96	2.80	160 g
C. *Slaughtering results:*				
Average weight when sold	(kg)	105	105	
Slaughter weight	(kg)	80.90	81.7	
Slaughter loss	(%)	-22.9	22.1	1.8%
Valuable parts of pork	(kg)	34.76	36.38	1.62 kg
Valuable pork of the progeny of 100 sows	(kg)	26105	29.213	3108 kg
Slaughter weight reduced to valuable pork	(kg)	61.52	62.66	
Slaughter weight reduced to valuable pork per progeny of 100 sows	(kg)	46202	50316	4114 kg
Relative output	(%)	100	108.9	8.9 %
D. *Economic evaluation:*				
Starch value requirement per 100 sows	(kg)	30798	30798	
Starch value requirement of progeny per 100 sows (to 30 kg)	(kg)	34141	36526	2485 kg
Starch value requirement of progeny per 100 sows during fattening	(kg)	166722	168630	2908 kg
Total Starch value requirement	(kg)	231661	235954	4293 kg
Relative input	(%)	100	101.85	1.85 %
Pooled relative economic index		100	106.9	6.9 %

superdominance and epistatic effects cannot be expected. Thus, it is not primarily from this point of view that the application of inbreeding is considered to be necessary.

b) Has inbreeding any role in the reproducibility of the obtained effects?

In our opinion this question can be answered in the affirmative. It is the very turning point of hybridization that insofar as any result above the average has been observed in a combination, this effect should be reliably reproducible. It is thought to be quite logical that in the case of combining inbred populations reproducibility is much more probable than in the cross-combinations of partners of wider gene frequency — i.e. of species. This explains, in fact, why inbreeding is considered to be unavoidable in pig hybridization too.

In the course of the work first the lines are developed by inbreeding (taking in consideration the results of blood-typing), and it is only then that the tests of various possible combinations begin. If any of the combinations prove suitable, the reproducibility of the effect can naturally be largely ensured. If, on the other hand, we start by testing a breed population, then establish the ranking of the combinations, the subsequent generations — when intercrossed — may not produce the same ranking order. Genetically this can be explained by a possible interference of dominance and epistatic effects with the result of the combinations of the two breeds. If, however, these genes are not manifested in the population, in the course of a continued breeding they may even be eliminated in the meioses, and thus will not be transmitted to the following generations. In this case combinations between the subsequent generations will no longer produce the effects observed initially. Naturally, this is only assumed on the basis of probability, but in planning the breeding work such considerations may play an important role.

c) Does the economic loss caused by inbreeding exceed the profitability of the end product?

The Western European geneticists generally maintain a negative attitude to this question. The breeders in those countries work with essentially smaller populations. Many breeders have to be convinced before a population not larger than on a medium large Hungarian farm can be bred on uniform principles. Their scruples are therefore easily understood. The situation is, however, different, if a smaller inbred population produces a large number of end products, which can be easily realized in Hungary. In the case of the KA-HYB the production of some 1 million hybrid pigs will be ensured in 1974 by 600 sows belonging to the inbred lines.

Speaking about the methodological questions of inbreeding the concept of "marginal depression" is the first to be mentioned. According to experience and when calculated with Wright's coefficient it is about $F_x = 0.15$. The individual tolerance of the animals largely depends on the extent of a homozygous state developed at the loci of their non-additive genes. Accordingly, depression may occur below $Fe = 0.10$, while in other cases it may not even be observed at 0.25.

Observations seem to prove that in pig hybridization Wright's inbreeding coefficient should only be taken in consideration as an informative figure, and not as the determinant of planning.

For example, the homozygosity assumed by the Wright-coefficient shows a probability of 0.25 both when two individuals of the same progeny and when a parent and its male or female progeny are mated. If, however, one or the other method is consequently applied in two or three successive generations, the progenies show considerable differences. It is so even if e.g. the male parent used in the parent-progeny mating is identical with the boar that had been the male parent of the first generation in the progeny-mating system.

After several generations the system of mating within the same progeny may result in new variants not sufficiently characteristic of either the initial male or the initial female parent. On the other hand, in the case of parent-progeny mating the following generations will more or less agree with the genotype of the individual by which the inbreeding has been carried out. All this means at the same time that when applying the method of parent-progeny mating less inbred generations are required to attain an adequate homozygous level than when mating males and females of the same progeny, so the former system is much more favourable even from the point of view of the time factor.

Further, in certain lines where the sixth to seventh generations are being produced and the inbred level is consequently $F_x = 0.10—0.12$ many more problems of depression are encountered today than in other lines with a much higher — even 0.25 — inbred level developed through fewer generations. In the case of such lines a higher value can — naturally — be set on conformity in a genetic sense.

It is a frequently observed phenomenon too, that in a litter produced by mating a male with its female progeny there are piglets whose viability and growth do not even show the least sign of inbreeding, while other piglets in the same litter show a high depression. In this case it is supposed that — although they are of the same litter — in some piglets a lower, while in others a higher homozygous level is brought about than that assumed by the Wright-coefficient. It would obviously be a mistake to take only the recorded inbreeding level of such litter-mates in consideration. Therefore — after eliminating the lethal gene effects — with a view to the supposedly high homozygous level male progenies showing a depression are given preference to the vigorous ones. Namely, supposedly the planned homozygosity has been better realized in the former. In such litters female piglets required for the maintenance of the line are chosen from the vigorous ones, while those showing depressions will be discarded. Namely, the further maintenance of the line would otherwise be jeopardized. At the same time we must be aware of the fact that these animals are not, in fact, at the homozygous level they are thought to be at on the basis of their herd-book data.

To sum up the above methodological observations, inbreeding is considered to be an indispensable means of pig hybridization. At the same time, it is important to point out that the term "inbreeding" is far from meaning a single scheme of solution; it resembles a musical instrument with many strings which offers various possibilities of playing.

Line breeding carried on with the purpose of pig hybridization requires a special way of selection too, which is not, however, identical with the one used at present in pure-breeding. In line breeding serving as the basis of hybridization the main point is to develop a genetic conformity as loci containing non-additive genes. It is thus evident that we must not take too many characteristics in consideration in the course of the matings if we are to advance at a reasonable rate toward the main objective.

The major characteristics influencing the economic production of pigs can be divided into three groups:

a) properties related with productivity and viability on the mother's side;

b) properties related with growth and feed conversion;

c) properties determining the quality of the slaughtered pig.

Those belonging to the first two groups are characteristics which — being of no, or only slightly additive nature — may be improved by crossing in proportion to the value of the combination.

On the other hand, it is known that as regards the properties related with the quality of the slaughtered product no improvement caused by the effects of crossing can be expected. They are transmitted in an intermediary way corresponding to the mean value of the joint properties of the parents.

Characteristics transmitted in a non-additive way usually have a low h^2-value.The lower the value of the latter, the more it represents characteristics that show a great depression as a result of inbreeding. They display, however, the most expressed effects in crossing. Intra-allele and interallele gene interactions playing a decisive role can mainly be expected in gene loci determining the value of these characteristics.

Characteristics with high h^2-values transmitted on the basis of additive gene effects show a different behaviour in the course of breeding. They are but slightly affected by depression caused by inbreeding, on the other hand, they are not found to improve by crossing. In crossing they show an intermediary course of transmission — just as in the case of pure breeding. As to the intermediary course of transmittance it must be noted that here we are speaking of a continuent intermediary effect, in contrast to the Mendelian intermediary transmittance known with the qualitative character where in the second and subsequent generations segregation can be observed.

Between these two groups showing different ways of transmittance are the characteristics whose h^2-values are medium high ranging from 0.2 to 0.3. They include first of all those influencing the growth vigour and the feed conversion. These characteristics are transmitted partly in an additive partly in a non-additive way, thus representing a transition between the two former groups. Consequently, these properties react to inbreeding with a lower degree of depression, while in the case of crossing may shown some effects — even if the latter are not too significant. Therefore in the case of these properties a simultaneous application of selection and testing for combinability seems to be desirable.

What has been said about the three groups of characteristics at the same time throw light upon the fact that selection made in pig hybridization has to be fully co-ordinated with the above aspects.

If the main point is to develop the genotypic conformity of non-additive properties within the line, and — in the second place — selective progress has to be made in the additive properties influencing the quality of the slaughtered product too, then there is no possibility left to make a simultaneous selection in the non-additive properties, too. This would jeopardize the improvement expected in the most important characteristics. Therefore, a compromise is needed at this point. For properties like resistance, fertility, piglet raising ability, growth vigour and feed conversion no selection is made within the line. Hybridization renders this possible, since the manifestation of these properties in the crossed progenies depends on nicking rather more than on the parents' qualities. The lower the h^2-value of a character, the more probable its realization. Therefore in the selection of lines the average level of the qualities of the initial breed is taken as a basis. According to our experience — though depression caused by inbreeding may result in a deterioration of the members of the line — when they are used for crossing this does not cause any disadvantage. Namely, with these properties two factors may be added up in the crossing: one of them is the crossing effect — which is not the same in every case —, the second is the transmitting ability of the partners. The latter generally manifests itself in proportion to the h^2-value characteristic of the various qualities. These two factors jointly determine the non-additive characteristics of the crossed progenies.

B) *Production of synthetic paternal lines*

As referred to in the course of discussing the production of pure-bred paternal lines immigration is an indispensable way of maintaining highly inbred lines consisting of a relatively small number of animals. One of the possibilities of immigration is to introduce individuals valuable for their additive properties from another breeding stock of the same initial breed. The other possibility is to use animals belonging to a different breed. This does not represent any danger to the maintenance of the homozygous state as far as the dominance of the line can be preserved. This can be attained by mating the animals originating from another breed, — and later their progenies too — with the most inbred members of the line. As long as the origin of such immigrated animals is deliberately changed, this serves as a way of introducing fresh blood in the line. It may occur, however, that — as a result of certain conditions — special — synthetic — lines are produced. For example, on the "November 7" Co-operative Farm at Balatonszabadi a male progeny with quite unusual muscles was born by mere chance in one of the lines. The typically four-hammed pig was set to breeding as soon as possible, and mated with sows (from lines I, II, III, IV, VII) which came nearest to this type. Unfortunately the original boar died after one year, still some 60 litters originated from it. From these litters we selected the piglets which inherited the same extent of muscularity. In the first generation minor faults — like small frame, short and low body, etc. — were overlooked in many of the animals. The best males were appointed as stock boars of the line. Partners were chosen for them on the basis of a mating scheme, using back-crossing to the outstanding quality ancestor in every case. From the thus produced larger second generation individuals could be sorted out which, though showing the original muscularity, did not meet the other requirements. The presence of more than one line — through the mothers — ensured in this population a genotypic variance sufficient to carry out a strict selection. By now a valuable synthetic paternal line (marked "CX") has been developed, which contains a considerable number of animals with a muscle system similar to that of the Pietrain breed, but with an about 8—10 cm longer body, larger frame and no pigment. As a particular value, it possesses the muscularity of the most muscular breeds in the world: the Pietrain and the Belgian lowland pig, without being related to them. It is, therefore, a highly suitable crossing partner for them which explains the great interest shown by the western breeders. In this synthetic line a continuous intensive inbreeding to the initial individual was applied, so today it is sufficiently homogeneous. Its main advantages include that it combines the great body length with an unusually lean muscle system.

The development of the paternal line "CX" presented as an example also seems to prove that the method of synthetic line breeding enables the amalgamation of outstanding features — transmitted mainly on an additive genetic basis — of animals belonging to different breeds, then their stabilization and reliable transmittance by subsequent inbreeding. The development of a synthetic line like that requires — according to our experiences — a population of about 200 sows, further, that the progenies of the other breeds utilized should serve the purpose of introducing fresh blood in the line over generations, without hindering the stabilization of the qualitative level of the required characters. In the cited example this was feasible in such a way that the best male progenies of the initial boar farrowed by sows from different lines were kept. In developing the subsequent generations, besides an inbreeding to the initial boar the alternating of the formers serves two purposes:

a) Inbreeding is only performed with the initial boar and not with its somewhat lower value partners.

b) A new immigration can be postponed to a later date after the development of the line.

Namely, in the case of certain synthetic lines which, e.g. are based on animals of quite extraordinary qualities it makes all the difference whether a new immigration — which is unavoidable if fertility is to be maintained — is carried but when the line has been genetically stabilized or when it is still at the stage of development.

Our experiences obtained with the method are summarized as follows:

a) it is of importance first of all in the case of additive character properties;

b) in the first generation after crossing those animals are to be kept which in the required property have inherited the prominence of their parents even if their other properties do not attain the expected qualitative level. Those animals which, though satisfactory in general, are not sufficiently typical in the property chosen as the main target to be attained, are less valuable from the point of view of breeding, considering the way the additive characteristics are transmitted. Although with the traditional way of selection such animals seem to be better than those possessing, e.g. enormous hams but at the same time short legs, still for the present method they are less suitable than the latter ones;

c) in the second generation after crossing, but only when a fairly large population is available which attains the planned level in the desired properties, the culling of animals which in some other aspect deviate from the standard may begin;

d) the desired genetic basis of the produced new population is stabilized by inbreeding, the genetic conformity in the non-additive characters is established;

e) when developing a synthetic line inbreeding should be performed with the founder of the line from the beginning, and not with its less valuable former partners;

f) immigration required for maintaining the fertility of the new line should be postponed by an adequate programming of the line build-up to a date when the genetic stability will have been ensured. In this way immigration no longer threatens to impair the quality of the already established characters.

From then on the line produced by this synthetic breeding method can be considered identical with any other population of non-synthetic origin.

C) *Development of F_1 paternal combinations*

The term F_1-combination is usually understood to mean the 50—50 gene percentage males of a line based on two different breeds. They are mostly produced for economic reasons. Important aspects are the relatively low number of inbred (i.e. expensive to maintain) animals required for the method, and the better viability, resistance and libido of the crossed males compared to the inbred ones. A disadvantage of the method is, on the other hand, that special combining ability, heterosis effects are more difficult to attain than when using pure-line males. There are a great many pig types in the world. There are full-bred bacon-type pigs, and pig types with extraordinary wide musculature which at 100 kg slaughter weight show the best indices of meat yield. Further, there are pigs representing various transitional forms between the two. The pig types can be divided again according to their demands into groups highly, medium and less reactive to the ecological conditions.

It is not a single "standard hybrid type" that we want to develop in our breeding work; we are endeavouring to produce animals meeting the most diversified requirements. Accordingly we have developed lines which may fulfil a special demand for bacon-types, but also ones able to realize maximum requirements by their excellent muscle system. There are variations adaptable to various intensive managemental conditions which at the same time serve the main purpose of our work: a more economical production by pig hybrids.

After these preliminary remarks let us point out that we consider the "tandem-system" combination of inbred paternal lines to be the most feasible way of producing F_1 combination paternal lines. The development of these tandems is determined by two factors:

a) combining ability between two lines, then between the combination of the two and the partner;

b) the economic type and its relation with the market demand.

In the former case decision is made exclusively on the basis of test results. As pointed out before, special combining ability between a paternal line of two breeds and a maternal line similarly of two or more breeds can hardly be expected. According to our experiences a more or less expressed crossing effect can be obtained in this case, but heterosis — based on inter- and intraallele gene interactions — only seldom, and if so, its reproducibility is an even more difficult task.

Such possible tandem groups are:

In bacon-type	In ham-type	In other type
Sweedish landrace	Pietrain	Hampshire
Danish landrace	Belgian landrace	Lacombe
English landrace	KA-HYB "CX"	German landrace
Sweedish large white	KA-HYB CXI.	English large white
	KA-HYB CXIII.	

There are, however, demands raised by the market for maximum maternal productivity (battery piglet factories). Others seek for a pig type with a top rate of growth and feed conversion. Such are,e.g. the fattening-farms which are in partnership with the piglet factories. Their interest lies in the most economical fattening of the delivered animals so as to attain the required live weight in the shortest possible time.

The tandem-system production of hybrid paternal lines is able to realize these objects too. In such cases the combinability tests of the lines give an answer to the question which of the F_1 paternal combinations are able to attain outstanding results in a certain priority feature. In our own practice several years experience is available concerning the different lines. For example:

Lines transmitting the highest fertility: II/A, III, IV, VII, XI, L.
Lines transmitting the highest growth vigour: II/A, IV, XI, L, LIII.
Lines transmitting a special muscularity: I, XLIX, LI, LIII, CX, CXI, CXIII.
Lines transmitting the best pork quality: II, IV, XLIX, CX.
Lines transmitting the strongest constitution: L, II, VII, XLIX, L.

The results of extensive tests performed so far enable efficient occasional transformations of the individual lines into tandems. On the other hand, it is a piece of luck that certain lines are simultaneously counted among the best transmitters in other very important properties, too. So — to cite the former example — a piglet factory with two thousand sows supplying 7—8 fattening plants with piglets raises a double demand:

a) for a maternal line showing the highest possible fertility and piglet raising ability, since the economic efficiency of pig breeding in such a plant is decisively determined by these qualities.

b) It has to produce piglets with the highest possible growth rate and feed conversion, as the economical production of its partners decisively depends on these qualities.

In this special situation it is of particular importance that the lines marked II/A, IV, XI and L are by far superior to the other lines in transmitting both properties. The four partners are in themselves suitable for the rapid production of a hybrid for this special purpose.

It requires consideration whether to develop paternal tandems from different types — for example from an extreme bacon-type and a similarly extreme ham-type. Namely, in this case opposed effects — therefore compensating each other — can be expected in the combination even if the crossing effect were in itself favourable.

As regards the additive characters the attainable results can be calculated in advance. In the case of the non-additive properties, on the other hand, which fundamentally influence the economical production of pork, negative surprises may even occur. We refer here to the statement that as a result of crossing the crossing effect and the inheritability of the property in question are added up.

The advantages of the method of F_1 male production are thus:

a) The production costs are much lower, as a smaller number of inbred animals have to be maintained. The cost of raising males excluded for some reason from breeding means a smaller loss to the producing unit than in the case of inbred boars.

b) As to viability, resistance, readiness to mate and fertility the F_1 boars are superior to the pure-bred ones. This holds true especially of the representatives of the "four-hammed" type, in which difficulties arising in the mentioned properties are much greater than in the case of other types.

The disadvantages are:

a) With the F_1-combination paternal lines, when coupled with maternal lines of more than one breed, true heterosis effects — superdominance, epistasis, etc. — are more difficult to attain than with pure-line males.

b) The same applies not only to the effects of superdominance or epistasis, but also to the simple crossing effect.

c) In the practice of pig hybridization a system in which the breeding farms buy hybrid sows only once will in all probability be the most feasible. The mothers of the following generation will subsequently be chosen from the fattening stock. By choosing an adequate paternal line from them a method built on the basic principles of hybridization and ensuring a satisfactory result can be elaborated, which will be described when discussing the development of maternal lines. It requires, however, many more paternal lines, the maintenance of an appropriate gene reservoir, and a continuous testing. The tandem-system use of males depletes this gene reservoir at a double speed which raises a number of further genetic problems.

II.

Hybrid maternal lines

The development of maternal lines for the KA-HYB hybrid pig is based on different methodological principles than that of he paternal lines. While in the case of the paternal lines we try to attain the highest possible concentration of the non-additive gene basis, with the hybrid maternal population the aim is the opposite. Its development is based on the genetic principle that the crossing effect depends in the first place on the extent to which the non-additive gene pairs of the individuals become homozygous. Since there is supposedly a greater possibility of attaining this aim—with a repeated and systematically performed combination, the methodology of sow production has been elaborated from this point of view.

The inbred level of the maternal — initial — basic line may reach the limit of depression, that is ranges between 0.10 and 0.15 F_1 values from the beginning of the work. In the first three or four generations produced by us the general productivity of the line remained at the average level characteristic of the breed. However, in the subsequent generations the non-additive genetic variance was greatly reduced in spite of the fact that the inbreeding coefficient was left unchanged, and this resulted in a decreased productivity. We endeavoured to balance the situation thus developed by establishing an incentive price level and using high nutritive value feed concentrates.

We are justified in mentioning the latter in this place by the fact that the depression caused by inbreeding increases to a great extent the sensitivity of the animals to their environment.

Owing to the fact that as a result of inbreeding the buffering capacity has become labile, increased demands are raised towards managemental factors. In improving our initial maternal line a good service was involuntarily done by the fact that the gene centre of this population had to be changed on several occasions. These changes took place in such a way that from the former place selected, high quality young animals were transferred to the new place. This almost forced out a faster rate of succession of the generations with all its advantages. This line originally had a stock boar named FENIX for its founder, but later its progeny of the second generation called 8. FENIX became the centre of the line. In its time this boar showed the highest fattening test results in Hungary. Of the sow stock initially originating from more than one family the sows Nos 1, 77 and 127 — in which the blood of the original FENIX was multiplied, and which excelled by far in fattening ability — played a decisive role in the further development of the line.

At present the sixth–seventh own-bred generation of the line is being produced, in which the original concept of breeding and the goals of selection have remained the same. All present members of the line are inbred to 8. FENIX, and the mentioned excellent sows were the mating partners of the boar 8. FENIX.

From the methodology outlined below it follows that this population is becoming superfluous as a basic maternal line and will be transformed into a paternal line. Still we thought it necessary to discuss it in detail, since this line was the very first link in our entire work of pig hybridization, and will play an important role in the future too.

This basic maternal line was combined with three inbred partners: XI, IV and V. The female progenies born from these combinations were tested for maternal productivity. The evaluation of the first two farrows of each of at least 400 sows, and of the related characters seems to provide a reliable basis for judging a cross-combination. The most favourable of the examined alternatives was reproduced, then the first progeny was further tested with new inbred partners. The results of the two-step series are given in Figs 1, 2.

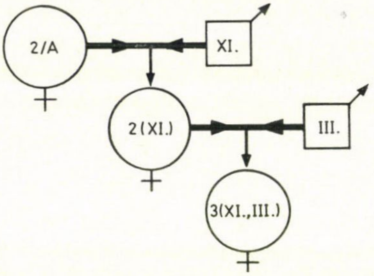

Fig. 1. Genetic construction of one of the maternal lines of KA-HYB (standard type)

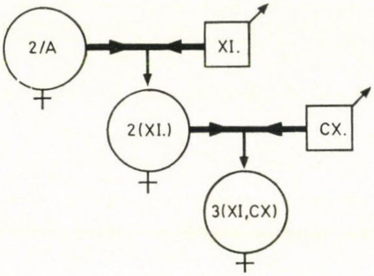

Fig. 2. Genetic construction of the maternal line of the KA-HYB super-ham type

Both are three-line maternal populations. Of the components participating in their development the paternal lines are always highly inbred (Wright $F_x = 0.25$—0.37). In the first years these maternal combinations marked 3 played the role of the mother for the end product.

Increasing the maternal productivity is one of the most essential points in pig hybridization, as it is of greater importance from the point of view of economic efficiency than either the improvement of the unit output, or feed conversion, or even the slaughter quality.

The capacity of the hybrid pigs can be increased in two ways:

1. By a recurrent selection of permanent character combinations — produced from few lines — those individuals will be given a more important role in the basic population whose progenies most frequently produce outstanding crossing effects. Namely, in the case of such progenies special intra- and interallele gene interactions are assumed to be produced in the combination of the partners. This means that the parents of these crossed animals have such genes at their gene loci which in combination with a partner endowed with the same gifts induce such interactions. With a continued regular selection we can increase the number of individuals in the basic population which in the case of certain combinations may realize these — highly desirable — effects in their progenies to a greater extent than earlier.

2. Instead of the above outlined — undoubtedly slow — method we may try to find other, more efficient combinations by introducing more than one line. The latter method will by all means reduce the possibility of obtaining special gene effects — if it does not make them perfectly impossible. (It should be noted, however, that there are but limited possibilities of obtaining special gene effects even in the case of the four-line hybrid, particularly when F_1 combination males are used for them.) The prospects of obtaining a sound, intensive crossing effect — not based on superdominance and epistasis — are according to our experiences much better. This is one of the compromises frequently encountered in the work of breeding and only avoidable in theory.

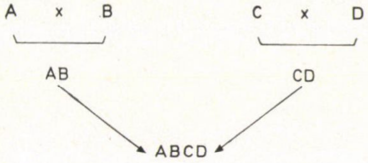

Fig. 3. Classic formula of the discontinuant hybridization

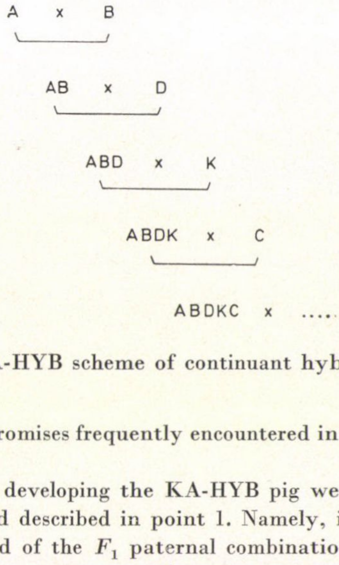

Fig. 4. KA-HYB scheme of continuant hybridization

In the methodology of developing the KA-HYB pig we follows the second procedure without discarding the method described in point 1. Namely, in most cases pure-line inbred paternal lines are used instead of the F_1 paternal combinations.

In developing the maternal lines we follow the genetic principle that the higher the crossing effect in the hybridization the more non-additive gene pairs become heterozygous in the sows producing the end product. The crossing effect can be best observed in respect of the resistance and the maternal qualities — conception, fertility, progeny rearing. Our method is therefore of special importance in the development of the maternal lines. An increased heterozygosity more probably occurs in repeatedly crossed maternal lines than in the case of a single-cross combination. The necessity of knowledge and purposefulness in producing these combinations are, naturally emphasized.

In this case the population of combination "AB" plays the mother's role for the end-product. Selection is also determined by the maternal productivity manifest in the population, inasmuch as in the "A" and "B" populations too the role of the individuals whose female progenies indicate the existence of special gene effects by their production records will subsequently be dominant.

The second part of the investigation consists of finding out what the fattening ability and slaughter quality of the end-product will be if the maternal population marked "AB" is crossed with the "CD" or other combination paternal line.

The scheme of development in the breeding methodology of the KA-HYB pig differs from the cited classical model.

The difference between the two is shown first of all by the fact that the hybrid maternal lines are in this case the results of repeated combinations — planned in advance and based on tests —, while the hybrid paternal lines are always inbred, pure-line populations.

This method offers a better chance for realizing the idea of ensuring the crossing effect most spectacularly demonstrable in the maternal properties by a nearly optimum heterozygous state in the non-additive features. A great advantage of this method is its economicalness. Each breeder sow is assumed to produce five new sows a year, and each hybrid sow twenty porkers. The production of the hybrid sows exceeds by far that of the pure-bred ones; this is one of the most important points in our work. In fertility this means some 20 per cent, while in the annual number of weaned piglets about 10 per cent superiority. There are differences in the percentage less of sows too, which also has a considerable economical influence.

When hybridizing after the classical model 2000 pure-bred sows have to be maintained to obtain 10 000 hybrid sows, and from them 200,000 end-products a year. In the KA-HYB system, on the other hand, only 400 pure-bred sows are required to attain the same result. As to the economic efficiency of production it makes an essential difference whether the grandparents on the mother's side are pure-bred, or already crossed, themselves. If they are pure-bred, this means — according to our model — four thousands less piglets a year, with only a single factor taken into account. If — and this would be, in fact, the aim in such a case — the blood of the parents of individuals showing special gene effects becomes dominant in the initial populations, then the possibilities of selection for productivity in the line will be still more restricted, so it will fall back even compared with a normal pure-bred population, because in the latter selection can be made for it as well.

In our own methodology in the course of time we have partly given up — based on these considerations — to develop superdominance or epistasis by the continuant maintenance of an F_1-construction hybrid maternal population. In the case of maternal lines we produce instead, multiple — but purposefully-crossed maternal populations at a much more favourable input cost and in larger numbers, naturally with a selection aimed at increasing the heterozygous state of the non-additive gene pairs. The pure-line and inbred paternal lines replacing the F_1 males have had — in our practice — a more concentrated influence on the end-product as well as on its phenotypic uniformity. This method means at the same time certain genetic recompensation too. Namely it almost gives the same chance of obtaining high value effects as in the case of applying the classical model, only the centre of action of the components is transferred.

After these preliminary remarks we present here our methodology of producing hybrid maternal lines (Fig. 7).

The origin of the sow population "4" shown in Fig. 7 can thus be traditionally represented as follows.

This method of producing maternal lines may be called continuant hybridization. It has two essential components:

1. It enables a better approximation of the optimal heterozygosity of the hybrid maternal lines — in the non-additive gene pairs —, with all the known advantages.
2. The evaluation of the crossing effect is not built — as in the traditional method — on the result of crossing all partners with one another, but decidedly on the nicking between the inbred paternal lines producing the successive generations.

According to our experiences it is not the same, even in this case, whether we work with crossed, pure-bred but not inbred, or with inbred paternal lines.

In practice this means, in fact, that in the concept of producing KA-HYB continuant maternal lines the importance of the extreme maternal origin is pushed into the background. The combination of the two paternal lines used in the last step always plays the decisive role. A large amount of experience gained in our twelve years work is available concerning the favourable combinations.

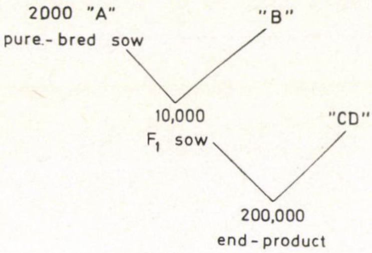

Fig. 5. Distribution by breed of the sow stock in discontinuant hybridization

When using F_1 paternal lines the situation is different. Quite naturally the possibility of clearly distinguishable nicking — already reduced from the mother's side — decreases here. It is caused by the large number of participant lines playing a practically equal role in developing the end-product.

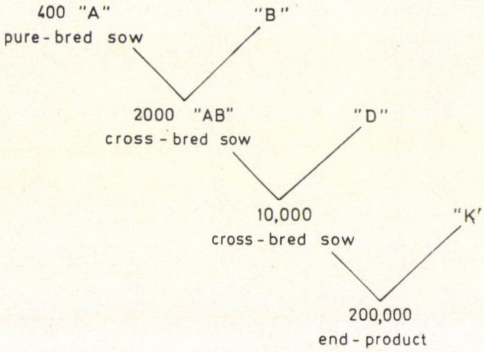

Fig. 6. Distribution by breed of the sow stock in continuant hybridization

The use of pure-bred but not inbred tarna results in a more favourable situation than that brought about by the application of F_1 paternal lines, because in this case pure-bred males are used instead of males originating from the combination of two partners. Since, however, the genetic variability of a breed is always higher than that of an inbred line, the expected dominance of the combination is not sufficiently ensured yet.

Our work is built in the first place on cross combination tests performed with the inbred paternal populations. As the homozygosity of their genotype creates an aspect more favourable for us, its manifestation in the genotype of the progenies is apparently more expressed.

The main point of the system of producing hybrid maternal lines — as seen in Fig. 7 — is that the female individuals of the population originally planned to be the end-product are regularly subjected to testing including their maternal performance.

This method occasionally renders the detection of new qualities possible which may exceed by several tenths the performances of the previous maternal line. Taking environment genotype interactions in consideration the results of the first and second farrows of at least 400 sows per poulation are analyzed. This covers:

- the number of losses in sows,
- the percentage of conception,
- the number of piglets born,
- the number of piglets at the age of 21 days,
- the progeny raising ability of sows.

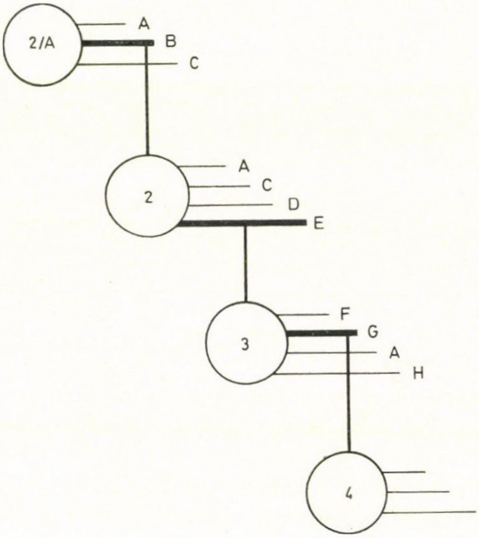

Fig. 7. Model of test and continuity

If any of the cross-combinations are proved to be better than the previous ones, their systematic reproduction is the following step. The process of reproduction takes about two years. During these two years the female progenies originating from the very first farrow are subjected to similar tests, so by the time the previous line is put into commercial use we know whether a further step is possible with them, and if so, what paternal line will be the most suitable mating partner for the next generation.

According to our experiences gained so far the maternal performance of the successive generations has not in any case proved worse than the previous one, but many of the variations have not been better. In other cases, again, while the maternal performance of such new maternal combinations exceeded the performances of their mothers, the quality of the end-product born from them did not represent any improvement, that is, suitable partners had not been found for them. In such cases we had to stop.

The practice developed in the course of this work seems to prove that the utilization of the successive end-product generations as female parents is theoretically possible. This can be continued as long as by mating them with suitable paternal lines we are able to produce populations somewhat exceeding, or at least reaching the level of the previous generations. In practice this means that until a certain optimum heterozygous state sets in, the method may result in animals with better qualities than the previous ones. If in such a work an adequate gene reservoir is available, the genetic "limit" can be considerably extended. However, until this point is attained new maternal constructions can parallelly be produced which in a given case may assume the role of the earlier ones for further 10—15 years. In pig hybridization it would be a great mistake to build everything on a single "construction". It is, therefore, advisable to work with more than one parallel variation — concerning their type and crossing effects — to be able to follow the constantly changing demands of the market.

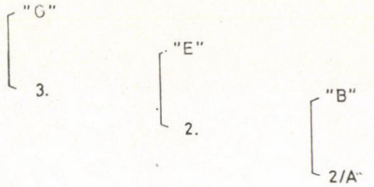

Fig. 8. Origin of the maternal line No. 4 on the basis of Fig. 7

Every new "would-be" maternal line is studied from three aspects:

1. From the aspect of its own maternal performance. This includes their physical strength and capacity as mothers. This is to some extent distinguished from their combining ability with other paternal lines.

2. Tests are performed to find out what male partners are able to produce an end-product best meeting the requirements of economicalness and the demands of the market and whether they are able at all to produce such progenies.

3. Which is the paternal line by which progenies suitable to produce a new sow generation can be obtained. It is not always identical with the partner that ensures the most favourable end-product. In the latter case we also examine the economic properties to be reckoned with in the course of raising the "would-be" sows and the other members of the litter until they reach a live-weight of 110 kg.

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ROLE OF STEM–FRUIT RELATION IN THE AFTER-RIPENING PROCESS OF RED PEPPERS

The red colour of red pepper berries is significantly influenced by the time of harvest, stage of fruit ripeness and time and method of after-ripening.

Comparisons of various methods of after-ripening have revealed that a significantly higher pigment content can be attained when the berries are left on the cut off stem during the period of after-ripening than when they are removed from the stem before after-ripening.

Table 1

Pigment content developed by after-ripening in red, sooty and green berries of the "Csokros csüngő" red pepper variety

Harvesting time	Pigment content g/kg					
	Red		Sooty		Green	
	−	+	−	+	−	+
Beginning of September						
1968	3.80	5.03	4.29	5.48	3.22	4.50
1969	5.79	7.21	5.29	7.14	2.68	4.56
1970	5.82	7.10	5.47	7.18	4.10	6.12
1971	7.81	8.49	6.92	8.88	2.72	6.48
1972	—	8.62	—	6.88	—	5.07
Mean	5.81	6.96	5.49	7.17	3.18	5.42
%	100	120	100	131	100	170
Mid-September						
1968	4.11	5.48	3.94	4.34	2.68	4.01
1969	5.97	7.38	4.72	5.36	1.89	3.02
1970	5.80	6.70	5.86	6.81	3.42	5.59
1971	7.24	7.65	5.70	5.75	2.52	4.61
1972	7.81	7.13	5.20	5.22	2.80	2.66
Mean	6.19	6.87	5.08	5.50	2.66	3.98
%	100	111	100	108	100	150

Notes:

— = The connection between stem and fruit was broken during after-ripening; after-ripening of removed berries

\+ = The stem-fruit relation was maintained during after-ripening; after-ripening on removed stems

The less ripe the otherwise fully developed berry the higher the influence of stem-fruit relation on the pigment content developed by after-ripening. In the pigment formation of unripe fruits during after-ripening the relation with the stem is decisive. On the after-ripening of red-ripe fruits, on the other hand, the root system has a favourable effect.

The ground product of the red pepper fruit-walls is a spice speciality. Its value lies in the first place in its red colour. The Hungarian red pepper is world-famed for its flame-red colour and excellent flavour. The world-wide reputation is ensured by the first-rate varieties and favourable climatic conditions of Hungary. Beyond these two factors the conditions of processing — especially the after-ripening of the berries — decisively influence the colour and quality of the end-product.

Table 2

Pigment content in the ground product obtained from the total yield (joint amount of red, sooty and green berries) of "Csokros csüngő" red repper plants in the case of different harvesting times and after-ripening methods (1972)

Harvesting time	Pigment content g/kg	
	After-ripening of removed berries	After-ripening of berries on the removed stem
At the beginning of September	4.62 = 100%	6.40 = 139%
Mid-September	5.02 = 100%	5.78 = 115%
At the beginning of October	5.10 = 100%	5.13 = 101%

In a generally used method the berries are removed from the stem before submitted to after-ripening for 4—6 weeks (Vinkler 1971). Compared to the pigment level thus developed a significantly better result can be attained, however, by the method of "After-ripening on the cut off stem" elaborated at the Horticultural Research Institute (Angeli—Sasvári 1971, Sasvári—László 1972). Experience shows that a more favourable pigment level can be developed by after-ripening when the connection between stem and fruit is maintained than

Table 3

Variance analysis of factors influencing the pigment content (g/kg) of red, sooty and green red pepper berries

Factor	FG	Red MQ	Sooty MQ	Green MQ
Total	63			
Replications	3			
Main effects:				
Time of harvest	1	19.3***	97.5***	13.5***
Period of after-ripening	1	0.1	1.6	0.4
Way of after-ripening	3	5.2**	3.5*	4.6***
Primary interactions:				
Harvesting time and after-ripening time	1	9.5**	4.8*	0.4
Harvesting time and way of after-ripening	3	2.9	3.4*	4.7***
Time and way of after-ripening	3	8.7***	0.6	2.1*
Harvesting time, time and way of after-ripening	3	2.2	3.1*	0.8
Error	45	1.2	0.9	0.5

when it is discontinued. Berries after-ripened on the cut off stem will have a higher pigment content than those removed from the stem (Tables 1—2).

As no detailed information was available on the influence exercised by the stem–fruit relation on pigment formation we started an experiment to find out how and to what extent berries of various ripening stages left on the stem were influenced by the other parts of the stem: foliage, shoots, roots. The investigations covered other factors acting on pigment formation too, namely: the time of harvesting and the period of after-ripening.

The starting point of after-ripening was determined by the following three stages of ripening: the red berry was the ripest, the so-called "sooty" berry medium ripe and the green berry the least ripe. Within each group of ripening the effect of the time and way of after-ripening on the pigment content was studied in four replications.

Table 4

Effect of harvesting date and after-ripening time on pigment content developed by after ripening in the red, sooty and green berries of "Csokros csüngő" red pepper plants (pigment content g/kg)

Harvesting time and ripening stage	Period of after-ripening		Effect of harvesting time
	4 weeks	6 weeks	
Earlier harvest (beginning of September)			
Red	8.0^{ab}	8.7^{ab}	8.4
Sooty	6.5	7.4	6.9
Green	3.9^{f}	3.6^{f}	3.8
Later harvest (mid-September)			
Red	7.7^{a}	6.7	7.3
Sooty	4.6^{d}	4.3^{d}	4.5
Green	2.8^{g}	2.8^{g}	2.8
Effect of the time of after-ripening			
Red	7.9^{c}	7.8^{c}	
Sooty	5.5^{e}	5.8^{e}	
Green	3.4^{h}	3.2^{h}	

Note: Between figures marked with the same letter there is no significant difference, but between unmarked pigment values belonging to the same ripening group there is a significant difference at least at P°

Harvesting was carried out on a single occasion, either at an earlier date (at the beginning of September) on later (in mid-September). In each case two periods of after-ripening were employed: a four-week and a six-week period. Both after-ripening periods were separately applied for each way of after-ripening. The following ways of after-ripening were used:

1. The connection between stem and berry ceased. The berries removed from the stem were after-ripened in net bags hung un a covered but not closed place.

2. The berries were left on the stem, but the stems were pulled out of the soil and laid one by one on the ground in rows. So the examined berries remained in connection with the other parts of the stem: berries of various ripening stage, foliage, shoots, with an inactive root system.

3. The berries were left on the stem. The totally defoliated stems having been pulled out of the soil were placed in rows on the ground. So the examined berries remained in connection with the other ones of various ripening stage, and with the shoots — with an inactive root system.

4. The berries were left on the stem, and the defoliated stems in the soil. So the examined berries remained in connection with other berries of various ripening stage, and with the shoots and the intact root system.

With each sampling the same number of berries was taken. The stage of ripening was established at the time of harvesting; the removed berries were placed in separate net bags according to their respective colours, and those left on the stem were marked in a similar way. Where the stem-fruit relation was maintained during after-ripening we endeavoured to pick berries of identical ripening stage from the same stem on each occasion of sampling, so as to ensure the same time of after-ripening.

Table 5

Effect of harvesting time and after-ripening method on the pigment content developed by after-ripening in red-, sooty- and greenripe berries of "Csokros csüngő" red pepper plants (pigment content g/kg)

Harvesting time and ripening stage	Way of after-ripening with the stem-fruit relation stopped maintained				Effect of harvesting time
	1	2	3	4	
Earlier harvest (beginning of September)					
Red	7.4^{ah}	8.5^{aj}	8.0^{ak}	9.7	8.4
Sooty	5.8	7.2^{c}	7.0^{c}	7.6^{c}	6.9
Green	2.4^{e}	4.8^{d}	4.4^{d}	3.5^{f}	3.8
Later harvest (mid-September)					
Red	7.0^{gh}	7.7^{gj}	7.2^{gk}	7.3^{g}	7.3
Sooty	4.5^{lm}	5.1^{l}	3.9^{m}	4.4^{lm}	4.5
Green	2.9^{en}	3.1^{n}	2.6^{n}	2.8^{fn}	2.8
Effect of the way of after-ripening					
Red	7.2^{r}	8.1^{st}	7.6^{rs}	8.5^{t}	
Sooty	5.1^{u}	6.2^{x}	5.5^{ux}	6.0^{x}	
Green	2.6	3.9^{v}	3.5^{vz}	3.2^{z}	

Note: Between figures marked with the same letter there is no significant difference, but between the unmarked pigment values belonging to the same ripening group (red, sooty or green) there is a significant difference at least at $P_5\%$. The ways of after-ripening 1—4 correspond to those discussed under "Material and method"

The pigment content was determined by Benedek's method. The tables show g/kg pigment content values. They mean the g quantity of total pigment content expressed in capsantine per 1 kg ground red pepper. The pericarp obtains its red colour from a higher quantity of capsantine and a smaller quantity of capsorubine (CHOLNOKY 1937, 1958). The pigment content values of the tables are always related to dry matter.

The experimental results were evaluated by variance analysis.

The variety used in the experiment was: "Csokros csüngő fűszerpaprika" (a rosette-type pendant red pepper variety).

The variance analysis of factors influencing the pigment content is shown in Table 3.

The effects of the harvesting time and after-ripening period are shown in Table 4 and that of the way of after-ripening in Table 5. From the data of the experiment the following conclusions can be drawn:

1. The time of harvesting has a significant influence on the amount of pigment developed with after-ripening in the fruit wall of the berry. An earlier harvest ensures a singificantly higher pigment level than a later one.

2. The way of after-ripening — the existence or non-existence of a connection between stem and fruit during the period of after-ripening — significantly influences the pigment content. The less ripe an otherwise fully developed berry, the more important the maintenance of stem-fruit relation during after-ripening. Regarding an earlier harvest and different ways of after-ripening, a significantly higher pigment content can be attained in sooty and green fruits by after-ripening on the removed stem. In the pigment formation of berries thus after-ripened the connection with the shoot system is the decisive factor. The foliage — as it soon withers and falls off — does not cause any significant change in the pigment content. The after-ripening of red-ripe berries left on the stem, on the other hand, is favourably influenced by the intact root system.

3. The four - and six-week period of after-ripening used in the experiment did not significantly influence the pigment formation. We have to note here that in analyses performed later taking a wider range of after-ripening periods in consideration the significant effect exerted on the pigment formation could be pointed out (LÁSZLÓ—SASVÁRI 1973). Namely, the curve of pigment content plotted as a function of the after-ripening period shows a maximum at five weeks of after-ripening. Therefore, three and five weeks of after-ripening — if taken instead of the four- and six-week period (before and after the maximum) — would supposedly have significantly influenced the pigment formation.

The highest pigment level would be attained if the red pepper berries were left on the stem until the pigment content value corresponding to the character of the variety developed. Since this is not made possible by the meteorological conditions, a nearly optimum result can be attained by maintaining the stem–fruit relation during the period of after-ripening.

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THE INITIAL PHASE OF TRAUMATOGENIC RESPIRATION

At the Research Institute of Precision Engineering, with the kind assistance of Mr. György Révész electrical engineer, head of department, we have constructed an instrument for special respiration measuring purposes. Any information on the technical details of measuring can be obtained for the time being from the above mentioned institute only, so we cannot touch upon this subject here.

The new methodology has made it possible to study the so-called traumatogenic respiration appearing after the injury of plant parts (Farkas 1968, Frenyó 1954, Rosenstock *et al.*, 1972) in an initial phase that so far has not been sufficiently accessible to investigations.

Although traumatogenic respiration is a phenomenon somewhat similar to parasitogenic respiration, as in both cases the compartmentization within the cell is expected to break up, we do not wish to lay special emphasis on the importance of the initial phase. The processes starting several hours after the injury and introducing the regenerative division, callus formation and suberification, respectively, are certainly far more important (Kahl *et al.*, 1970). If we were to categorize we might call the subsequent increased respiratory activity, most thoroughly studied so far, secondary traumatogenic respiration which is connected with a new protein or enzyme synthesis as the obvious consequence of the RNA synthesis preceding it.

It is e.g. unimaginable that the mentioned internal factors synthetize in the very moment of cutting a potato tuber, apple, kohlrabi, raddish or other plant parts of firm texture. Consequently, the almost instantaneous initial increase of respiration can be regarded as a primary traumatogenic respiration, as it is totally different both in its quality and inner conditions from the process that takes place much later. It was, in fact, only suspected or observed in a qualitative way that the cells reacted with an immediate increase of O_2 uptake to injuries. This had to be thought of, if only because of the observed brown oxidation of the polyphenols; Boswell and Whiting even attempted measuring in 1938 (Annal. Bot .N. S. 2,847), but measuring in 20 minute intervals could not, naturally, give information on what happened a few seconds after the injury.

As to our approach, instead of starting from the terminal oxidation we measured the CO_2 discharge of the injured surfaces, and that within a few seconds. This could be most easily attained by measuring the time in which a 1 cm^2 area of the injured surface discharged a known quantity — e.g. 50 microgram — of carbon dioxide. Such a small quantity of carbon dioxide is produced in a few seconds. We measured the speed of motion the pointer of the instrument's microammeter showed between two suitably chosen scale values. The same surface was measured on fifteen successive occasions. In the case of young potato tubers the following times were obtained: 11, 31, 40, 45, 45, 62, 54, 40, 56, 39, 65, 47, 57, 55, 65 seconds.

A 24 second preparation time must be taken into account before each measuring. It must be declared emphatically that the great fluctuation of the numbers is not by any means the standard deviation of the error as proved by the fact that when measuring the respiration of intact tissues we obtained a fluctuation lower by orders of magnitude.

The above series of numbers shows the extraordinary speed of CO_2 discharge — in essentials of decarboxylation — immediately after the cutting. It seems as if a repression has ceased. The second measuring gives evidence of an almost three times lower respiration rate. Then, with a higher or lower fluctuation, between 39 and 65 seconds, the discharge of the same amount of carbon dioxide continues. That is, the initial rate of respiration shows a decreasing tendency, then within 2 hours comes close to the respiration of an intact tuber through the same surface area of the undeveloped epidermis (75 seconds). After several hours the secondary traumatogenic respiration characteristic of the process of regeneration, well-known from the literature, begins.

We showed the course of primary traumatogenic respiration intentionally in a single tuber instead of giving the average result of several parallel experiments. Namely, the equalization of the individual fluctuations might suggest that after the sudden initial increase the abnormal traumatogenic respiration takes an undisturbed course of decrease, although the processes taking place in the plant tissue, or at least on the surface of the wound, can with a slight exaggeration be called dramatic.

The initial phase of the traumatogenic respiration — while showing different values — had perfectly the same course in the storing organs of all plants examined so far. It may be mentioned, however, that the respiration is more intensive in parts closer to the epidermis and rich in plasm than in cells with larger vacuoles farther in. This, otherwise not unexpected observation agrees with the investigation results of KAHL *et al.* (1970). It is hardly disputable that the almost sevenfold instantaneous increase of the intensity of respiration compared to that of intact tissues can be traced back to the effect of cutting which destroys the established inner structure in a cell layer. It remains to be examined, however, how far the partial change of pressure occurring as a result of cutting contributes to the unusually rapid release of carbon dioxide. The possibility of the so-called disconnection cannot naturally be left out of consideration either.

Although the preliminary report cannot explain every phenomenon, it can be supposed that at the end of the exceedingly intensive initial phase of the traumatogenic respiration the respiration of intact layers so far repressed by the carbon dioxide released from the destroyed structures will prevail. The rapidly dying cells then play no further role, so a somewhat more uniform respiration can be observed until the outset of the secondary processes.

By means of an instrument of special construction we measured the so far unknown initial phase of traumatogenic respiration in plant parts (tubers, fruits) cut open. The first seconds are characterized by a considerable release of carbon dioxide suggesting the termination of a repression and — perhaps — a phenomenon of disconnection, beside the breaking up of compartmentization.

The phenomenon can be regarded as the primary phase of traumatogenic respiration which shows a decreasing tendency with high fluctuations.

The new increase of respiration some hours later — which has been treated at length in the literature — is considered to be the secondary phase of the traumatogenic respiration.

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NEW HOST PLANTS OF THREE ISOMETRIC PLANT VIRUSES

Within the research programme of the virus-host relationship we made investigations into further host plants of the radish mosaic virus (R/ * : */ * : S/S : S/Cl, **Mamula** *et al.* 1972) and turnip yellow mosaic virus (R/1 : 1.9/34 : S/S : S/Cl, **Horváth** *et al.* 1973, **Juretić** *et al.* 1973) recently isolated in Hungary. We extended our experiments to include a strain of tobacco ring spot virus of American origin (R/1 : 1.8/42 : S/S : S/Ne) kindly placed at our disposal by Dr. J. W. **Demski** (Georgia Experiment Station, Experiment, Georgia, U. S. A.).

In the course of our experiments we examined the following virus-host relations: radish mosaic virus — *Brassica carinata* A. Br., *Cucumis myriocarpus* Naud., *Gomphrena decumbens* Jacq., *Solanum rostratum* Dun., *Tetragonia crystallina* L'Hérit; turnip yellow mosaic virus — *Brassica carinata**, *Cucumis myriocarpus*, *Gomphrena decumbens*, *Solanum rostratum*, *Tetragonia crystallina*, *Tinantia erecta* (Jacq.) Schlechtend.; tobacco ring spot virus — *Cucumis myriocarpus*, *Gomphrena decumbens*, *Phaseolus acontifolius* Jacq., *Phaseolus mungo* L. (results concerning these two latter species were already published by **Cheo—Zaumeyer** (1952), *Tinantia erecta* and *Tetragonia crystallina.*

Our findings concerning the virus susceptibility of plants included in the experiments — except the two *Phaseolus* species — can be regarded as quite new in the literature on plant virology.

In the experiments we employed artificial inoculation methods with the carborundum-spatula technique known in the practice of plant virology. The radish mosaic virus and turnip yellow mosaic virus were propagated in *Brassica rapa* L. var. *rapa* plants; the inoculum required for the infection was provided by the tissue fluid of these plants. Virus infection both in the inoculated and newly formed (non-inoculated or subsequently developed) leaves of the examined plants was established by virus re-isolation tests. In the case of the above two viruses *Brassica rapa* var. *rapa* plants were used for this purpose. The tobacco ring spot virus was propagated in *Nicotiana tabacum* L. cv. *Xanthi*-nc, a systemic host plant, and its tissue sap served as inoculum containing virus. The re-isolation of the tobacco ring spot virus from the plants to be examined was carried out with *Phaseolus vulgaris* L. cv. *Red Kidney* plants.

In the course of studying the responses of *Brassica carinata* inoculated with radish mosaic virus and turnip yellow mosaic virus we found that this plant was locally and systemically susceptible to infection by both viruses. Leaves of plants inoculated with radish mosaic virus showed mild symptoms of vein clearing, while on the leaves formed after the infection systemic

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* We carried out investigations on the susceptibility of *Brassica carinata* to turnip yellow mosaic virus because the notable handbooks (e.g. **Klinkowski** 1968, **Schmelzer—Wolf** 1971, **Smith** 1972) only mention it as an experimental host plant; no reference can be found to symptoms caused by turnip yellow mosaic virus.

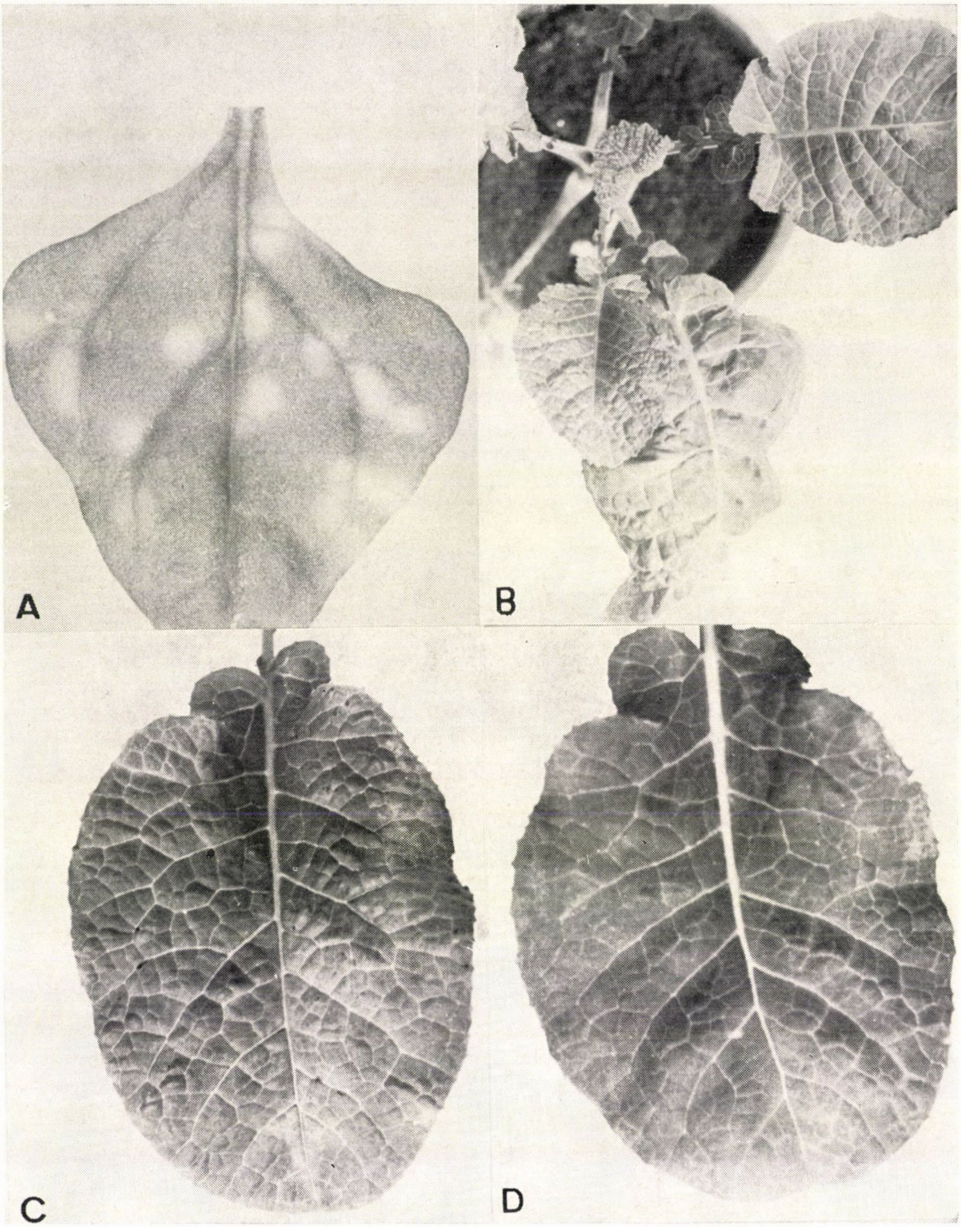

Fig. 1 Inoculated leaf of *Tetragonia crystallina* L'Hérit infected with radish mosaic virus (A). Systemic symptoms induced by radish mosaic virus in *Brassica carinata* A. Br. (B and C). Systemic leaf symptoms of *Brassica carinata* A. Br. caused by turnip yellow mosaic virus (D)

vein clearing, crispness, blistering, then scattered tiny, black, necrotic spots appeared. In some cases a slight vein necrosis was also observed. The inoculated plants showed an intensive growth inhibition (Figs 1B and C). On the inoculated leaves of plants infected with turnip yellow mosaic

Fig. 2 Symptoms caused by tobacco ring spot virus in *Phaseolus acontifolius* Jacq. (A and B) and *Phaseolus mungo* L. (C and D) plants. A and C are local, while B and D systemic symptoms

virus vein clearing, and in many cases large chlorotic spots appeared. On the non-inoculated leaves typical ochre mosaic spots developed, which are characteristic symptoms of the turnip yellow mosaic virus on its main host plant, the turnip.

On the inoculated leaves of *Tetragonia crystallina* infected with radish mosaic virus large chlorotic spots appeared (Fig. 1A). Systemic symptoms of the virus could not be demonstrated. The virus re-isolation tests also pointed out a local host-virus relation between the *Tetragonia crystallina* and the radish mosaic virus. The *Tetragonia crystallina* proved to be resistant to the turnip yellow mosaic virus. Similar resistance was shown by the *Cucumis myriocarpus*, *Gomphrena decumbens*, *Solanum rostratum* and *Tinantia erecta*. The first three plants were found to be resistant to the radish mosaic virus as well.

In the course of experiments performed with tobacco ring spot virus the infected *Phaseolus acontifolius* and *Phaseolus mungo* plants were found to be susceptible to virus infection both locally and systemically. According to earlier experiments by Cheo—Zaumeyer (1952) the above two plants proved also locally and systemically susceptible to infection by the tobacco

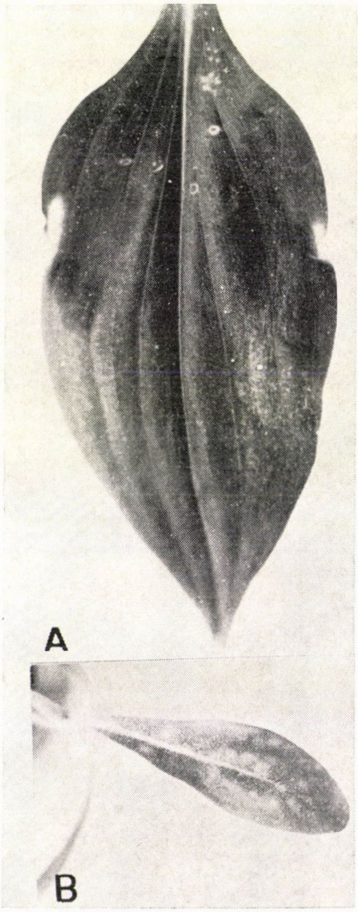

Fig. 3 Local symptoms caused by tobacco ring spot virus in leaves of Tinatia erecta Jacq. (A) and *Gomphrena decumbens* (Jacq.) Schlechtend. (B) plants

ring spot virus. In our experiments on the inoculated leaves of the above two *Phaseolus* species large, necrotic, mostly concentric rings developed (Figs 2A and C). On the non-inoculated leaves severe chlorotic, then necrotic lesions appeared followed by a systemic total apical necrosis (Figs 2B and D). A similar local and systemic disease was induced by the tobacco ring spot virus in *Tinantia erecta.* The symptoms developed in this plant were, however, essentially milder (Fig. 3A), consisting of tiny grey concentric lesions. Apical necrosis causing total destruction could not be found in any of the cases. *Cucumis myriocarpus, Gomphrena decumbens* plants inoculated with tobacco ring spot virus did not develop even local symptoms either. On the inoculated leaves of *Cucumis myriocarpus* chlorotic rings appeared 8—10 days after the infection; the virus did not become systemic in the infected plant. On the inoculated leaves of *Gomphrena decumbens* chlorotic, then rust-coloured necrotic lesions developed (Fig. 3B). On non-inoculated leaves no symptom appeared; in spite of this the virus could be re-isolated from the newly developed leaves. *Solanum rostratum* showed a latent local and latent systemic susceptibility to infection by tobacco ring spot virus. The *Tetragonia crystallina* reacted by chlorotic spots, a spottedness extending to the axillary shoots too, as well as leaf deformation and leaf necrosis, as systemic symptoms to infection by the tobacco ring spot virus.

In the course of our experiments we found two further host plants *(Brassica carinata, Tetragonia crystallina)* to radish mosaic virus. *Brassica carinata* can be regarded as a new virus host plant to turnip yellow mosaic virus, too. Among the new host plants of tobacco ring spot virus *(Cucumis myriocarpus, Gomphrena decumbens, Solanum rostratum, Tetragonia crystallina, Tinantia erecta)* special attention should be paid to *Tinantia erecta (Commelinaceae)*, which in our earlier investigations proved to be susceptible not only to alfalfa mosaic virus (R/1 : : 1.3/18 : U/U : S/Ap) but also to tobacco mosaic virus (R/1 : 2/5 : E/E : S; Horváth 1970, unpublished). We mention here that *Tinantia erecta* gives local and systemic responses to infection by alfalfa mosaic virus. Both on the inoculated and non-inoculated or subsequently developed leaves red-brown irregular necrotic spots appear which in most cases start from the main rib. Red-brown necroses can be found on the internodes, too. *Tinantia erecta* has a symptomless susceptibility to tobacco mosaic virus. According to our investigations made so far *Tinantia erecta* is resistant to potato aucuba mosaic virus (*/ * : */ * : E/E : S/Ap), potato virus X (R/1 : */6 : E/E : S/[Fu]), potato virus Y (*/ * : */ * : E/E : S/Ap) and turnip yellow mosaic virus. According to our experiments *Tetragonia crystallina* can be used as a screening plant for the differentiation of radish mosaic virus and turnip yellow mosaic virus.

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LEAF AREA AND ITS COMPONENTS IN SPRING BARLEYS

It is known that the most important physiological processes, e.g. respiration, photo synthesis and the regulation of the water regime, which play a decisive role in the life of the plant and the amount of its yield take place in the leaves. Soil, water and plant form a dynamic system (Idso 1968) The total dry matter yield including the grain and straw yield as well as the root remains is determined by the size of the assimilating surface and by the photosynthetic activity (Vavilov—Kabüs 1970). Both features are characteristic of the variety but their extent depends on the ecological conditions.

The importance of the leaf area had already been recognized earlier, and various methods were described for its determination Pollhamer 1967, Rowley—Rasmusson 1969, etc.). The photosynthetic intensity was studied mainly with the so - called Warburg technique by Lowes—Treharne (1971), Berdahl—Rasmusson—Moes (1972), Danilchuk—Jatzenko (1972) and Felippe—Dahle (1972). It was proved that the bulk of the material contained in the grain was incorporated between earing and maturing (Birecke *et al.* 1967, Walpole—Morgan 1971). Further, a temporary shading—particularly of the uppermost leaf at the time of earing — was found to reduce considerably the photosynthetic activity and the dry matter production (Willey—Holliday 1971, Dale—Felippe 1972, Dale—Felippe—Fletcher 1972). After this many researchers began to measure the area of the uppermost leaf and study its role in the photosynthesis (Thorne 1961, 1965, Lawes—Treharne 1971, Tovmasyan 1971, and others). Simpson (1968) found a close positive ($r = +0.96$) correlation between the two characters. Apel—Lehmann (1970), on the other hand, only pointed out a low ($r = +0.36$) positive correlation. Others found no correlation at all. Subsequent experiments revealed that the development of a leaf area characteristic of the variety depended on many factors (Luebs—Lanng 1969, Kirby 1970, Lazarov 1971, Melville—Lanham 1972). Rowley—Rasmusson (1969) published data concerning the hereditary nature of the leaf area.

In spite of a relative abundance of new results few data are available on the importance of the leaf properties in breeding (Nátr 1972), and on the possibilities of selection. It has not been clarified either what the optimum leaf area and leaf type under Hungarian conditions are.

At Martonvásár the year 1973 was unusual for the spring barley. Our treatments were sown in well prepared beds until 16—19 March. Germination was satisfactory in spite of the fact that the amount of precipitation was only 4.2 mm in March. Barley lived on the 60 mm rain fallen in April almost until harvesting. Namely, in May the amount of rainfall was only 0.7 mm, and on the first ten days the average daily air temperature exceeded 20 °C. Some late varieties could make use of the June rainfalls, but the early varieties could not. In spite of all this the average grain yield of the spring barley varieties checked on 18th July was 50.01 q/ha.

Table 1

Leaf-width of 34 spring barley varieties at the time of earing, cm
Martonvásár, 11 June 1973

Variety	cm width of the									Variation coefficient %
	1st	2nd	3rd	4th	5th	6th	7th	8th	Mean	
	leaf from the top downward									
Mv 31	0.8	1.2	1.3	1.1	0.7	0.5	0.4	0.3	0.7	142.8
MK 42	0.6	1.2	1.2	0.8	0.6	0.4	0.4	0.3	0.6	150.0
MKH 23	0.6	1.1	1.1	0.9	0.7	0.5	0.4	0.3	0.7	114.2
Certa	0.6	1.1	1.1	1.3	0.7	0.4	0.3	0.2	0.7	157.1
Maris Mink	0.4	1.3	1.1	1.1	0.8	0.6	0.4	0.3	0.7	142.8
MKS 99	0.6	1.1	1.1	0.9	0.6	0.4	0.3	0.2	0.6	150.0
MKS 43	0.6	0.7	1.2	0.8	0.6	0.5	0.5	0.3	0.6	150.0
Willa	0.6	1.2	1.2	0.7	0.5	0.4	0.4	0.4	0.6	133.3
Maris Concord	0.5	0.9	1.0	0.8	0.5	0.4	0.3	0.3	0.6	116.6
MKS 50	0.5	0.9	1.0	0.8	0.6	0.5	0.4	0.4	0.6	100.0
MK 305	0.5	1.0	1.1	0.9	0.6	0.4	0.4	0.4	0.6	116.6
MKS 34	0.5	0.9	1.0	0.8	0.5	0.4	0.5	0.6	0.6	100.0
MKS 45	0.6	1.1	1.1	0.7	0.5	0.4	0.4	0.3	0.6	133.3
Inis	0.4	0.8	1.0	0.8	0.6	0.4	0.3	0.3	0.5	140.0
Midas	0.5	1.0	1.0	0.9	0.8	0.6	0.4	0.4	0.7	85.7
FD-054-30-3	0.5	0.9	1.0	0.7	0.5	0.4	0.3	0.3	0.5	140.0
MKH 81	0.3	0.9	1.1	0.6	0.6	0.4	0.3	0.2	0.5	180.0
Rika II.	0.5	1.0	1.0	0.6	0.5	0.3	0.2	0.2	0.5	140.0
MK 293	0.5	1.2	0.6	0.8	0.5	0.4	0.3	0.2	0.5	200.0
MK 311	0.5	0.8	0.9	0.7	0.5	0.4	0.3	0.3	0.5	120.0
MKS 29	0.6	1.0	1.0	0.5	0.5	0.5	0.4	0.2	0.5	160.0
MKS 59	0.6	1.0	1.0	0.6	0.5	0.4	0.4	0.3	0.6	116.6
Mk 307	0.6	1.0	0.9	0.6	0.5	0.4	0.3	0.3	0.5	140.0
Felda	0.5	1.0	0.9	0.8	0.5	0.4	0.4	0.2	0.5	160.0
Drossel	0.5	1,0	1.0	0.6	0.4	0.4	0.3	0.3	0.6	116.6
MKS 22	0.4	0.8	1.0	0.7	0.7	0.4	0.4	0.4	0.6	100.0
Carina	0.5	0.9	1.0	0.8	0.5	0.5	0.4	0.3	0.6	116.6
Imber	0.5	0.7	0.9	0.7	0.5	0.5	0.3	0.3	0.5	120.0
Carmen	0.5	1.0	0.8	0.6	0.5	0.4	0.3	0.2	0.5	160.0
MKS 26	0.3	0.6	0.8	0.8	0.5	0.4	0.4	0.3	0.5	100.0
Tern	0.4	1.0	0.9	0.6	0.4	0.4	0.3	0.3	0.5	120.0
Mona	0.6	1.0	0.9	0.6	0.4	0.4	0.3	—	0.6	116.6
W. Wing	0.5	0.7	0.8	0.6	0.3	0.3	0.3	0.3	0.4	125.5
Amkar T 444	0.5	0.9	0.9	0.6	0.3	0.3	0.3	0.2	0.5	140.0
Mean	0.5	0.9	0.9	0.7	0.5	0.4	0.3	0.2	0.5	—
Var. coeff.	100.0	77.7	77.7	114.2	100.0	75.0	100.0	200.0	105.5	132.5
Average of 23 autumn barleys	1.0	1.4	1.4	1.1	0.8	0.5	0.4	—	0.94	—
Var. coeff. %	50.0	28.0	42.8	63.6	75.0	80.0	75.0	—	44.4	112.4

For the purpose of investigations 34 spring barley varieties were chosen. With the earlier published method (Pollhamer 1967) the width and length of the first eight leaves downward from the top were measured on 25 plants per variety. On the basis of the obtained data the individual and average leaf area of each variety and leaf level, and the total leaf area per stalk for each variety were calculated. At the same time we calculated and expressed as the average percentage of the varieties the variation coefficients of the average leaf width and length, as

Table 2

Leaf-lengths of 34 spring barley varieties at the time of earing, cm
Martonvásár, 11 June 1973

Variety	Length of the									Variation coefficient %
	1st	2nd	3rd	4th	5th	6th	7th	8th	Mean	
	leaf from the top downward, cm									
MK 301	8.3	16.6	22.6	28.7	25.4	18.9	14.7	13.3	18.5	110.2
MK 42	8.9	17.3	22.9	28.3	27.3	19.9	14.5	12.9	19.0	102.1
MKH 23	7.5	17.3	25.9	25.1	24.1	18.8	14.8	11.1	18.0	97.7
Certa	8.6	16.4	21.6	27.7	25.2	17.5	12.8	12.1	17.7	107.9
Maris Mink	6.3	14.1	18.2	22.8	25.1	23.5	14.8	12.9	17.2	109.3
MKS 99	7.6	16.4	21.7	26.5	25.6	19.9	15.4	12.6	18.2	103.8
MKS 43	7.8	13.7	17.2	26.3	27.5	22.2	15.4	10.7	17.6	111.9
Willa	7.8	17.2	22.8	25.5	22.8	16.0	12.2	8.7	16.6	106.6
Maris Concord	7.7	16.5	21.4	27.7	28.2	19.8	15.7	13.8	18.8	109.0
MKS 50	7.9	15.5	22.1	24.5	23.1	18.5	13.8	9.7	16.8	98.8
MK 305	8.6	16.4	21.2	22.5	21.2	15.8	14.5	8.9	16.1	86.3
MKS 34	7.3	15.0	20.2	24.4	23.1	18.4	14.5	12.5	16.9	101.1
MKS 45	8.5	16.5	22.9	24.1	18.7	16.6	11.4	10.0	16.0	97.5
Inis	7.3	15.3	19.2	26.3	26.3	19.5	13.8	12.3	17.5	108.5
Midas	6.1	11.3	14.4	18.8	20.7	18.5	12.9	11.1	14.2	102.8
FD-054-30-3	7.9	15.5	20.7	25.5	23.9	16.9	15.0	12.3	17.2	102.3
MKH 81	6.2	13.9	20.6	26.0	25.9	17.9	16.3	13.9	17.5	113.1
Rika II.	6.9	15.1	18.6	25.9	29.7	21.4	14.9	12.3	18.1	125.9
MK 293	6.7	14.0	19.7	24.8	25.3	19.2	25.9	13.8	17.4	106.8
MK 311	8.8	18.3	24.1	24.4	19.9	15.4	14.8	11.8	17.1	91.2
MKS 29	8.7	15.8	21.6	26.2	19.7	14.1	11.9	9.4	15.5	112.9
MKS 59	6.4	16.0	19.8	24.4	22.8	16.0	11.7	9.9	15.8	113.9
MKS 307	10.3	18.1	23.1	22.9	18.8	14.5	11.6	10.9	16.2	79.0
Felda	8.6	18.3	21.8	26.3	23.9	17.2	15.9	12.0	18.0	98.3
Drossel	7.9	16.3	21.6	22.6	19.1	14.4	10.8	10.0	15.3	96.0
MKS 22	6.2	13.5	18.4	22.9	22.2	16.5	13.3	10.8	15.4	108.4
Carina	6.0	13.5	18.6	21.2	20.6	16.8	13.1	10.5	15.0	101.3
Imber	6.3	13.5	18.9	24.4	23.0	17.4	11.8	11.3	15.8	114.5
Carmen	8.6	15.5	20.8	21.8	19.7	15.5	11.6	9.2	15.3	86.2
MKS 26	4.5	11.5	16.9	22.0	24.0	20.6	13.8	11.5	15.6	125.0
Tern	6.4	13.8	19.1	23.4	20.6	16.2	12.5	9.8	15.2	111.8
Mona	10.3	18.3	20.3	18.2	14.7	12.7	8.8	—	14.7	78.2
W. Wing	6.8	13.5	18.9	22.3	20.0	17.6	13.3	12.8	15.6	99.3
Amkar T 444	6.7	14.0	18.7	23.7	19.6	13.6	11.0	10.2	14.6	116.4
Average cm	7.5	15.4	20.4	24.3	22.8	17.5	13.4	11.0	16.5	—
Var. coeff. %	77.3	45.4	56.3	43.2	65.7	61.7	55.9	45.4	56.3	103.9
Average of 23 autumn barleys, cm	12.2	21.1	22.2	21.4	17.7	13.9	11.8	—	17.2	—
Var. coeff. %	45.9	35.5	28.2	29.9	46.3	44.6	80.5	—	44.5	60.4

well as of the total leaf area per stalk relative to the varieties and to the individual leaves of the same leaf horizon.

After earing (4th June), then one and two weeks later (11th and 18th June) the above characters were measured and the values calculated on 25 plants of each of nine spring barley varieties. The weekly data of the per plant leaf areas of the varieties were plotted for comparison. The total and the active leaf area per plant were determined separately.

Table 3

Leaf area per leaf and per plant of 34 spring barley varieties at the time of earing, cm²
Martonvásár, 11 June 1973

Variety	Area of the								Leaf area, cm²		Var. coeff. %
	1st	2nd	3rd	4th	5th	6th	7th	8th	Total	Mean	
MK 301	3.4	10.5	14.9	16.5	9.6	4.7	2.9	2.1	64.8	8.1	177.7
MK 42	2.9	10.7	17.0	12.1	9.6	4.8	2.9	2.4	62.1	7.7	189.6
MKH 23	2.3	9.9	15.0	12.4	9.1	5.4	3.4	2.0	59.9	7.4	175.6
Certa	2.9	9.1	11.9	18.3	9.1	4.0	1.9	1.2	58.5	7.3	234.2
Maris Mink	1.3	9.5	10.5	12.7	10.1	7.7	3.2	2.0	57.4	7.1	160.5
MKS 99	2.4	9.4	12.2	12.4	7.9	3.2	2.6	1.4	51.9	6.4	171.8
MKS 43	2.4	5.3	10.7	11.4	8.8	6.3	3.9	1.8	50.9	6.3	152.3
Willa	2.4	10.5	13.7	9.2	6.3	3.6	2.6	2.0	50.6	6.3	185.7
Maris Concord	1.9	7.5	11.2	11.9	7.7	4.3	3.0	2.3	50.2	6.2	150.0
MKS 50	2.0	7.6	11.7	10.9	7.1	4.9	2.9	2.0	49.4	6.1	159.0
Mk 305	2.4	8.2	11.7	10.3	7.2	3.8	2.9	1.7	48.5	6.0	166.6
MKS 34	1.9	7.3	10.9	10.0	6.3	4.4	3.6	3.7	48.4	6.0	150.0
MKS 45	2.8	9.6	13.3	9.4	4.8	3.8	2.4	1.8	48.1	6.0	191.6
Inis	1.6	6.8	9.6	10.6	8.1	6.0	2.5	1.9	47.4	5.9	152.5
Midas	1.7	5.7	7.8	9.1	8.6	5.6	2.9	2.5	44.3	5.5	134.5
FD-054-30-3	2.2	7.4	10.3	8.9	6.1	4.0	2.8	2.1	44.1	5.5	149.0
MKH 81	1.2	6.8	11.7	8.0	7.7	3.5	2.5	1.7	43.5	5.4	194.4
Rika II	1.7	7.6	10.0	8.5	7.8	3.7	2.0	1.4	43.1	5.3	162.2
MK 293	1.8	9.0	6.1	10.5	6.8	3.9	3.2	1.5	43.1	5.3	160.3
MK 311	2.4	8.0	11.1	8.5	5.0	3.0	2.5	2.0	43.0	5.3	171.6
MKS 29	2.9	8.3	11.7	6.8	5.0	3.6	2.6	2.1	42.9	5.3	181.1
MKS 59	2.0	8.6	10.4	7.8	5.8	3.8	2.3	1.7	42.7	5.3	164.1
MK 307	3.1	9.1	10.6	7.9	4.7	3.2	2.0	1.8	42.7	5.3	166.0
Sv Felda	2.4	9.3	10.7	10.5	6.7	3.7	3.3	1.0	41.9	5.2	186.5
Drossel	2.2	8.5	11.5	7.5	4.6	3.2	1.8	1.5	41.1	5.1	196.0
MKS 22	1.3	5.6	9.4	8.7	6.5	3.8	2.9	2.1	40.5	5.0	162.0
Carina	1.6	6.5	9.4	8.8	5.1	4.2	2.6	1.8	40.4	5.0	176.0
Imber	1.6	5.3	8.5	9.1	6.5	4.4	2.3	2.2	40.1	5.0	150.0
Carmen	2.1	7.8	9.1	6.8	5.1	3.4	2.2	1.1	38.0	4.7	170.2
MKS 26	0.7	3.8	7.3	9.3	6.6	4.7	2.7	2.0	37.3	4.6	186.9
Tern	1.5	6.9	9.3	7.2	4.3	3.4	2.0	1.4	36.3	4.5	175.5
Mona	3.5	9.1	9.6	5.5	3.4	2.6	1.3	—	35.3	5.0	166.6
W. Wing	1.7	4.8	8.4	7.4	3.7	3.2	2.4	2.1	34.0	4.2	159.5
Amkar T 444	1.7	6.3	9.0	7.1	3.7	2.5	1.6	1.4	33.6	4.2	180.9
Average	2.2	8.1	11.1	10.0	6.8	4.2	2.7	1.9	45.7	5.7	—
Var. coeff. %	122.7	85.1	98.1	128.0	98.5	123.8	96.2	136.8	111.1	—	165.8
Average of autumn barley	6.6	15.7	16.1	12.9	7.7	3.7	2.6	—	—	9.4	—
Var. coeff. %	77.2	51.5	55.9	68.9	101.2	86.4	130.7	—	81.6	—	146.6

The relationship between the leaf area, the area of the uppermost leaf and the grain, straw and total yield was established by correlation calculations on the basis of the data of the per plant total leaf area of the varieties and of their yields attained in the experiments.

The examined 34 spring barley varieties developed an average of 8 leaves this year. Only the variety "Mona" had 7 leaves. The average width of the leaves was 0.50 cm. The second and third leaves from the top were found to be the broadest ones. Upward and downward from them the leaves gradually became narrower. The narrowest leaves were found at the base of the plant (Table 1). The differences between the varieties were negligible. The varieties MK 301, MKH 23, Certa, Maris Mink and Midas were found to be broad-leaved, while W. Wing was a narrow-leaved variety. The variation coefficients of leaf width for the varieties were higher than those for the leaf horizons. This year the average width of the uppermost leaf agreed with the average leaf width of the varieties, and thus seems suitable for characterizing it (Table 1).

The average length of leaves in the spring barleys is hardly shorter than that of the autumn varieties. Unlike the autumn barley, the fourth leaf from the top proved to be the longest of all (Table 2). Upward and downward from it the leaf length decreased. The fourth and fifth leaves of the spring barleys were longer than the leaves of the autumn barleys at the same horizon. The uppermost leaf, on the other hand, was the shortest of all, and it did not even attain half of the length of the upper leaf in the autumn barleys. Therefore, the length of the uppermost leaf in the spring barleys gives no information on the average leaf length per plant. Differences in leaf length between the varieties are rather considerable. With their more than 18 cm average leaf length MK 301, MK 42, MKH 23, MKS 99, Maris Concord, Rika II and Felda belong to the long-leaved varieties. The varieties Midas, Amkar T 444, Mona, Tern, Carmen, Carina and Drossel, on the other hand, are considered short-leaved varieties, owing to their average leaf length of 15 cm. The variation coefficients of the leaf lengths of the varieties are nearly twice as high as those determined for the leaf horizons. The variation coefficients of leaf length both per variety and horizon are substantially higher in the spring barleys than in the autumn barleys.

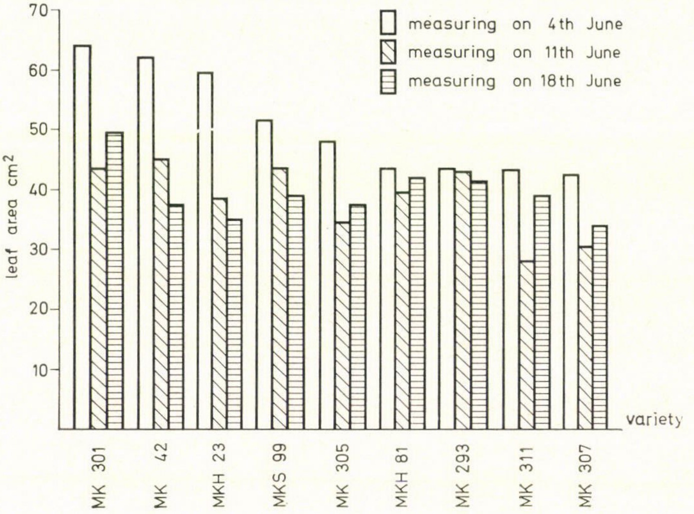

Fig. 1. Total leaf area per plant (cm^2) at the time of earing, and in weekly intervals after Martonvásár, 11 June 1973

The leaf area of the spring barley varieties was calculated from the leaf-width and leaf-length data of the varieties and leaf horizons (POLLHAMMER 1967). The per plant and individual leaf areas of the spring barley varieties were considerably smaller than the corresponding measurements of the autumn barleys (Table 3). The variation coefficient of the varieties was about one and a half times as high as that determined for the leaf horizons. The differences between the varieties were higher than those between the horizons. With their more than fifty square centimetre per plant leaf area the varieties MK 301, MK 42, MKH 23, Certa, Maris Mink and MKS 99 were considered large-surfaced varieties, while Carmen, MKS 26, Tern, Mona, W. Wing and Amkar T 444 proved to be small-surfaced varieties due to their less than 40 cm² leaf area per plant.

The third leaf from the top was of the largest area in the case of the spring barleys, too. Upward and downward from it the average leaf area gradually decreased. The lower- and uppermost leaves had the smallest average leaf area of all. The average area of the uppermost leaf does not give information on the average total leaf area of the variety. This conclusion is supported by the variation coefficients of leaf area per plant and per horizon, which in the case of the spring barleys are essentially larger than the corresponding values of the autumn barleys

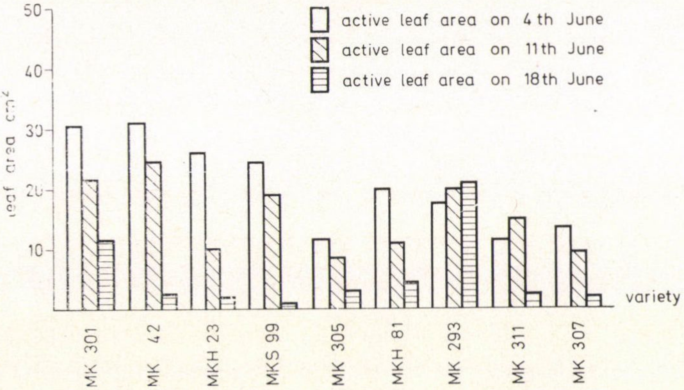

Fig. 2. Total leaf area per plant at the time of earing (cm²), and active leaf area per plant at the time of earing and in weekly intervals after (cm²). Martonvásár, 11 June 1973

The total per plant leaf area of the spring barleys (Fig. 1) — unlike the autumn barleys — was the largest at the time of earing, and gradually decreased in the course of subsequent measurements. It was remarkable that in varieties with a large total per plant leaf area this decrease was of a much greater extent than in those with a small leaf area. We were glad to find that the total per plant leaf area of the varieties MKH 81, MK 293 and MK 307 showed but a slight change. These varieties were fairly tolerant to infections by powdery mildew and earlier than usual drought.

In the case of autumn barleys all leaves were green and active at the time of earing, which cannot be said about the spring barleys. Due to an infection by powdery mildew and a very severe early drought the leaves began to turn into yellow from the base, then more or less wilted. At the time of earing the 5th to 8th, a week later the 4th to 8th, and two weeks later the 3rd to 8th leaves from the top downward became partially or totally incapable of assimilation. The area of the inactive lower leaves was subtracted from the total leaf area per plant to obtain the per plant active leaf areas of the varieties (Fig. 2).

As a result of the highly unfavourable conditions the active leaf areas of the varieties were only about half of the total leaf area already at the time of earing. The active leaf area continued to decrease, and two weeks later was only one-fifth of the leaf area found at the time of earing. The depressive action must have then been close to the critical value, because the per plant active leaf area of the varieties was reduced to nearly the same minimum value. As a very great advantage of the variety MK 293 its per plant active leaf area showed the lowest extent of decrease.

On the basis of the total per plant leaf area and the leaf area of the uppermost leaf in the examined 34 varieties, as well as of the values of grain, straw and total yield attained in the experiments the relationship between these characters was established with correlation calculation. It was only between the total leaf area per plant and the total yield per unit area that some positive correlation ($r = +0.46$) was found (Table 4). Between the other characters no correlation could be pointed out at all. We think, accordingly, that the area of the uppermost leaf is not sufficient to properly characterize the leaf area of a variety; we have to determine the total leaf area per plant. On the basis of the total per plant leaf area and the crop results, on the other hand, relatively small-surfaced, high productivity, drought tolerant varieties and strains can be selected.

Table 4

Correlations between the total per plant leaf area, the area of the flag-leaf and the grain, straw and total yield
Martonvásár, 1973

Character	Grain yield q/ha	Straw yield q/ha	Total yield q/ha
Total leaf area per plant, cm^2	$r = +0.05$	$r = +0.19$	$r = +0.46$
Area of the flag-leaf, cm^2	$r = -0.06$	$r = -0.08$	$r = +0.13$

*

Prepared at the Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár.

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NUMBER OF REPLICATIONS AND RELIABILITY OF THE EXPERIMENT IN WINTER WHEAT TRIALS

In the breeding work one of the aims of field experimentation is to study the the productivity of the different strains. The reliability of the experiment can be essentially influenced by two factors: the plot size (Balla—Szunics 1973) and the number of replications. By increasing the number of replications we can reduce the possibility of error. According to Sváb (1973) the larger the number of replications the more correctly the differences of treatments and the error of the experiment can be assessed. The above author recommends 4—6 replications for field experiments.

In general the reliability of the experiment is improved to a greater extent by the increase of the number of replications than by that of the plot size (Kudryavtzeva 1959, Molostov 1968, Dospehov 1968).

According to Dospehov (1968) the accuracy of the experiment considerably increases if the number of replications is raised to 6, but their further increase improves the reliability in a lesser degree. Similar results were obtained by Briggs—Knowles (1967). Salmon—Hanson (1970) do not recommend more than four replications.

Since the researchers have different views concerning the optimum number of replications, we considered a thorough study of this problem under Hungarian conditions to be necessary, taking first of all the efficiency of the breeding work into consideration.

The experiments were laid out at Martonvásár, at the Agricultural Research Institute of the Hungarian Academy of Sciences with 17 improved strains and a control variety (Bezostaya 1) in 1970 and 1971. The plot size was 15.6 m^2, the number of replications 18.

The soil was a medium sticky meadow loam, the forecrop a mixture of sunflower and pea. The amount of fertilizers applied was N_{120}, P_{100}, K_{80} kg/ha active agent.

To determine the accuracy of the experiment the following parameters were used: least significant difference (L. S. D.%) for which the probability level of the statistical decision was accepted — according to the international practice — at $P = 5\%$; variation coefficient (CV%), which generally can be accepted between 6 and 14 per cent (Sváb 1973). If the value of the variation coefficient is below 10 per cent then the variation is low, between 10 and 20 per cent it is medium, and above this high (Dospehov 1968). The error of mean value ($s_{\bar{x}}\%$). In field experiments an $s_{\bar{x}}$ value of 1—2 per cent is excellent, 3—5 per cent is good, 5—7 per cent satisfactory (Dospehov 1968). The closeness of the correlation and its significance was expressed by a correlation coefficient, while the regression correlation by the application of quadratic polynomial and hyperbola regression equations.

Table 1

Trends of L.S.D.$_{5\%}$, $s_x\%$ and CV% as a function of the number of replications (Martonvásár, 1970, 1971)

Parameter	Year	Number of replications						
		2	3	4	5	6	12	18
L.S.D.$_{5\%}$	1970	15.8	12.9	11.2	9.7	6.7	6.4	6.4
	1971	8.5	6.8	5.2	4.1	4.4	3.5	4.0
CV %	1970	6.5	6.8	6.6	6.9	5.5	7.1	8.3
	1971	4.2	4.4	3.8	3.6	4.7	4.9	6.7
$s_{\bar{x}}\%$	1970	4.6	3.8	3.3	3.0	2.3	2.0	1.5
	1971	3.0	2.5	1.9	1.5	1.9	1.4	1.6

The experiment was successful in both years, but in 1971 the examined parameters showed a higher accuracy (Table 1), and the values of L. S. D.%, $s_{\bar{x}}\%$ and CV % were — under the soil and climatic conditions of Martonvásár — similar to those in the previous years.

On the basis of the data obtained it has been established that with the increase of the number of replications the yield fluctuation decreases and the reliability of the experiment increases (Table 2). The values of L. S. D. % and $s_{\bar{x}}\%$ are, in essentials, of a similar trend, while the CV % does not show a uniform pattern. This must be due to a higher influence of the heterogeneity of the soil on this parameter. The accuracy of the experiment can be improved by an increase in the number of replications. An experiment with six replications gives satisfactory results in every year. In the case of favourable weather conditions a five replication experiment is also acceptable. According to our experiences less than five replications do not give satisfactory results. If the number of replications is reduced from six to three (a twofold reduction) the accuracy of the experiment decreases 1.5—1.9-fold, when it is reduced from six to two (a threefold reduction), then accuracy decreases 1.9—2.3-fold.

A too high number of replications (12 or 18) hardly improves the accuracy of the experiment. At the same time the area and the amount of seed required as well as the cost of the experiment substantially grow.

The data of the experiments show a significant correlation between the number of replications and the reliability of the experiment (Table 3, Fig. 1).

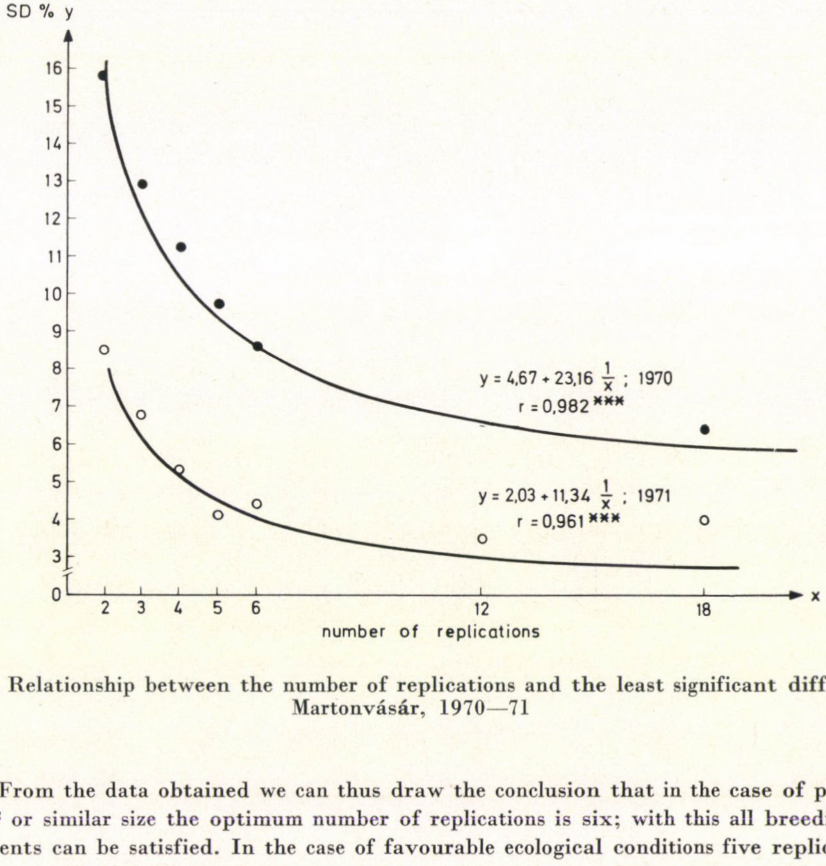

Fig. 1. Relationship between the number of replications and the least significant difference Martonvásár, 1970—71

From the data obtained we can thus draw the conclusion that in the case of plots of 15.6 m^2 or similar size the optimum number of replications is six; with this all breeding requirements can be satisfied. In the case of favourable ecological conditions five replications may be enough, if, however, we are to obtain precise results regularly, preference should be given to experiments with six replications. The application of more than six replications is neither necessary nor economical, and is only justified when the exposure is very high, frost damage, drought or pests can be reckoned with, and we know in advance that one or more replications will not be suitable for evaluation.

On the basis of our investigations we have arrived at the same conclusion as Kudryavtzeva (1959), Briggs—Knowles (1967), Molostov (1968) and Dospehov (1968). At the same time the maximum of four replications suggested by Salmon—Hanson (1970) is not sufficient under our conditions.

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Prepared at the Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár

L. Balla, L. Szunics

Table 2

Reliability of the experiment as a function of the number of replicatioons (%)

Parameter	Year	Number of replications						
		2	3	4	5	6	12	18
L.S.D.$_{5\%}$	1970	235.8	192.5	167.2	144.8	100.0	95.5	95.5
	1971	193.2	154.5	118.2	93.2	100.0	79.5	90.9
CV %	1970	118.0	124.2	120.0	126.0	100.0	128.9	151.3
	1971	89.5	92.6	81.0	76.8	100.0	102.3	140.7
$s_{\bar{x}}$ %	1970	199.6	167.1	144.7	131.1	100.0	85.9	66.2
	1971	157.4	132.4	102.1	81.9	100.0	74.5	83.5

Table 3

Correlation between the number of replications and the reliability of the experiment (in an experiment with 18 replications)

Parameter	Year	r	a	b_1	b_2	Regression equation
L.S.D.$_{5\%}$	1970	0.982	4.67	23.16		$Y' = a + b\frac{1}{x}$
	1971	0.961	2.03	11.34		
CV %	1970	0.821	6.99	—0.19	0.01	$Y' = a + b_1X + b_2X^2$
	1971	0.940	4.30	—0.09	0.01	
$s_{\bar{x}}$ %	1970	0.982	1.37	6.84		$Y' = a + b\frac{1}{x}$
	1971	0.943	2.51	3.48		

Note: the correlation coefficients are significant at P=0.1 %

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EFFECT OF B-995 (N-DIMETHYLAMINO SUCCINAMIC ACID) ON GROWTH, FLOWERING AND MINERAL ACCUMULATION OF TOBACCO PLANTS

A group of growth retarding chemicals has been described (Riddell *et al.* 1962) which reduce plant height effectively if sprayed on the leaves (Bukovac 1964, Cathey 1964). One of these compounds, N-dimethylamino succinamic acid (B-995) has been reported to modify the plant growth in size by decreasing the internode length in a variety of plants (Batjer *et al.* 1964). The objectives of this investigation were (a) to determine the effects of B-995 on growth, flowering and mineral accumulation and (b) to ascertain whether higher concentrations of B-995 than previously reported on other species of plants would cause significant changes in *Nicotiana tabacum* L. var. K-49 tobacco plants.

Nicotiana tabacum L. seed cultivars Kelieu-49 were sown in a seed-bed in a glass house and seedlings of uniform size and vigour, at the four leaf stage, were transplanted in cement pots (20"×14") containing a soil manure mixture with three seedlings per pot. After the seedlings had become well established, they were thinned out uniformly to one plant per pot. Forty-two days after transplanting, three concentrations of B-995, namely, 1250, 2500 and 5000 ppm were given by single foliar spray and one lot kept as a control. For better distribution and deposition of spray material, teepol (1 ml/L) was added as a wetting agent for all series.

Observations were recorded on plant height, stem elongation and on mineral accumulation at weekly intervals. The data on floral buds and inflorescence size were also recorded from the commencement of floral buds (40 days) after treatment.

Chemical constituents were analyzed for ash content (A. O. A. C. 1960), nitrogen by the micro-Kjeldahl method (A. O. A. C. 1960), phosphorus by (A. O. A. C. 1950), potassium by the cobaltinitrate method (Johnson—Ulrich 1969).

Growth responses: The effects of the B-995 sprays were beginning to be visible after about a week and differ significantly throughout the growth period. The effectiveness, however, differed according to the concentration of the solution applied, maximum retardation was caused by 5000 ppm of B-995. Lighter dosages were less effective (Fig. 1). Stem length was also influenced in the same way as the total height of the shoots with significant differences. It was also noticed that the reduction in both characters was well marked throughout the investigation period (Fig. 2).

The fresh weight of the stem was significantly decreased by B-995 applications (Fig. 3), and the maximum effect was observed at a concentration of 5000 ppm of the chemical. In general, the reduction in weight was less severe under the influence of lower concentrations but higher than the control. In the case of dry weight, the treatment effects were well marked but statistically insignificant (Fig. 4). The fresh and dry weights of the stem were relatively low in the beginning and increased with age for some time. At the maturation stage, there were again some decreases in these characters. The increase in stem weight is natural because of the growth. At the maturation stage, the centre foliage started drying and a reduction in the weight occurred.

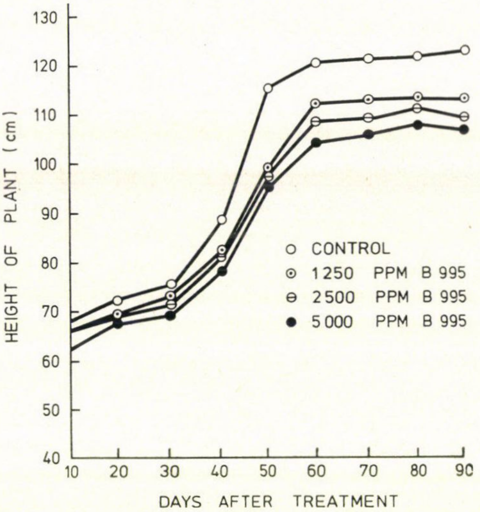

Fig. 1. Response of tobacco plants to foliar sprays of B-995 in plant height

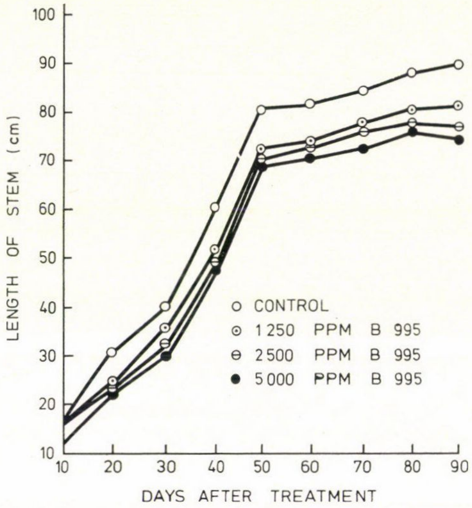

Fig. 2. Response of tobacco plants to foliar sprays of B-995 in stem length

Flowering and fruiting behaviour: Approximately 25—35 per cent of the plant population had developed the floral buds 40 days after the treatments. At this stage, the maximum number (31.7) was found in 1250 ppm treated plants followed by 2500 ppm (28.0) of B-995. On the other hand, the highest concentration (5000 ppm) gave 19.2 as compared to 26.7 in the untreated controls. However, the maximum number of flower buds was recorded at 60 days (Table 1), and statistically the effects were significant. Regarding the inflorescence size it was observed that the various concentrations of B-995 were found to be more effective than the

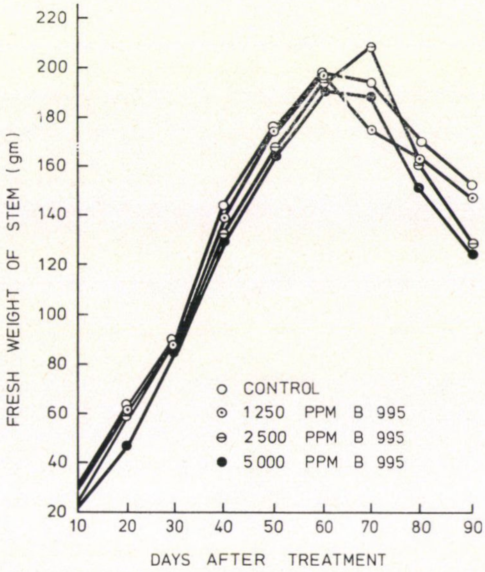

Fig. 3. **Response of tobacco plants to foliar sprays of B-995 in fresh weight**

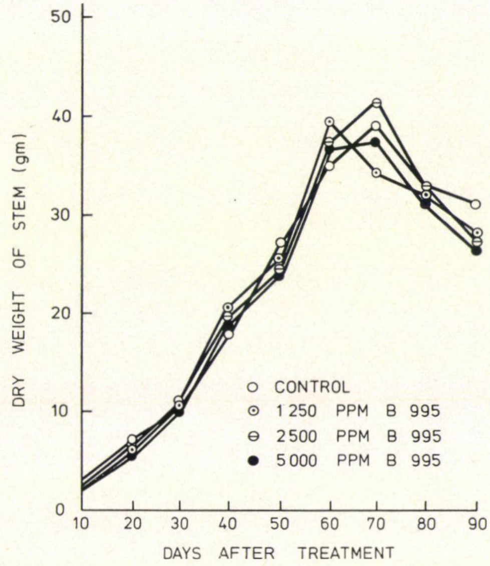

Fig. 4. **Response of tobacco plants to foliar sprays of B-995 in dry weight**

controls and differed significantly (Table 2). In general, 1250 ppm had the maximum effect on this character followed by 2500 ppm, the highest concentration (5000 ppm) showed inhibition.

Table 1

Average number of floral buds per untreated and B-995 treated tobacco plant recorded 40 days after treatment and the following 10-day intervals

Treatment	Days after treatment					
	40	50	60	70	80	Mean
Untreated (control)	26.7	64.0	78.0	29.0	10.0	41.14
1250 ppm B-995	31.7	66.0	80.5	30.2	18.2	45.32
2500 ppm B-995	28.0	63.2	92.5	30.2	12.7	45.32
5000 ppm B-995	19.2	65.7	91.2	25.0	10.0	42.22
Mean	26.40	64.72	85.05	28.60	12.72	

	Concentration	Age	Interaction
C.D. at 5% level	2.52	2.81	5.64
C.D. at 1% level	3.33	3.71	7.46

Table 2

Average length (cm) of inflorescence of untreated and B-995 treated tobacco plants recorded 40 days after treatment and the following 10-day intervals

Treatment	Days after treatment						
	40	50	60	70	80	90	Mean
Untreated (control)	13.7	39.1	45.7	51.2	46.2	46.0	40.31
1250 ppm B-995	20.7	40.0	55.2	58.7	56.5	56.4	47.91
2500 ppm B-995	19.4	30.7	53.2	52.7	48.5	48.5	42.16
5000 ppm B-995	14.0	29.8	48.5	52.0	47.7	47.2	39.86
Mean	16.95	34.90	50.65	53.65	49.72	49.52	

	Concentration	Age	Interaction
C.D. at 5% level	1.50	1.85	3.74
C.D. at 1% level	1.99	2.45	4.95

Table 3

Fruiting responses recorded on B-995 treated plants at the age of 90 days after treatment; mean 4 replicates

	Concentration in ppm			
	0	1250	2500	5000
No. of capsules/plant	138	210	193	168
Weight of seeds/plant (Gm)	10.38	11.77	11.27	11.16

Besides an increase in the number of floral buds and size of inflorescence, a marked effect on the fruiting behaviour was also noticed (Table 3). Fruits formed on the treated plants were more in number, depending upon the concentrations of B-995. The weight of seeds per plant was considerably superior in the treated plants relative to the controls. In general, 1250 ppm treated series gave better response on these characters. However, B-995 has a beneficial effect on flowering and fruiting in tobacco plants.

Mineral accumulation in the stem: The ash contents in the stems of the untreated and B-995 treated tobacco plants are presented in Fig. 5. 2500 ppm gave maximum content followed by the control whereas the highest concentration (5000 ppm) and the lowest concentration (1250 ppm) resulted in the lowest values. A higher nitrogen content had also accumulated in the stem than in the control when treated with B-995 (Fig. 6). Soon after treatment the nitrogen content increased considerably in the treated series but not in the controls. Later the values in all the treated plants including the controls gradually diminished but were always higher than in the control. In general, 2500 ppm showed a greater effect on the accumulation of the nitrogen content.

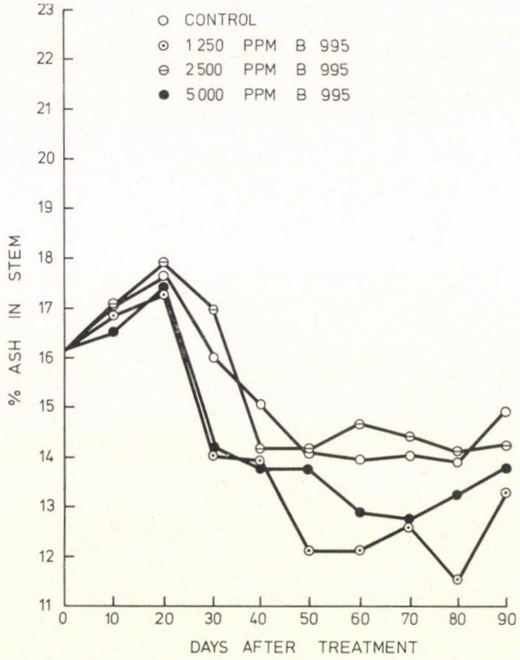

Fig. 5. Response of tobacco plants to foliar sprays of B-995 in ash

The phosphorus content in the stem of the treated plants was generally higher than in the controls (Fig. 7), and the maximum effect was noticed at 2500 ppm of B-995. During the later part of the experiment, however, the phosphorus content exhibited a somewhat decline in the 5000 and 1250 ppm treatments but the values, except for a brief period, were always higher than the control ones.

Regarding the potassium content, 2500 ppm of the chemical gave higher values, followed by 5000 ppm. On the other hand lower than control values were recorded for the 1250 ppm-treated series. However, the differences were not as prominent as those recorded for other elements (Fig. 8).

Plant growth was inhibited by the use of the growth retardant B-995 as evidenced by retardation in stem growth and weights. Maximum reduction was noticed at the highest concentration (5000 ppm). **Sachs** *et al.* (1960) reported a similar growth phenomenon and attributed it to the inhibition of cell division and cell elongation. **Mayr—Presoly** (1963) and **Yadava** (1970) reported that the growth retardants like cycocel significantly decreased the weight rather than length and this was correlated with an increase in cell wall thickening.

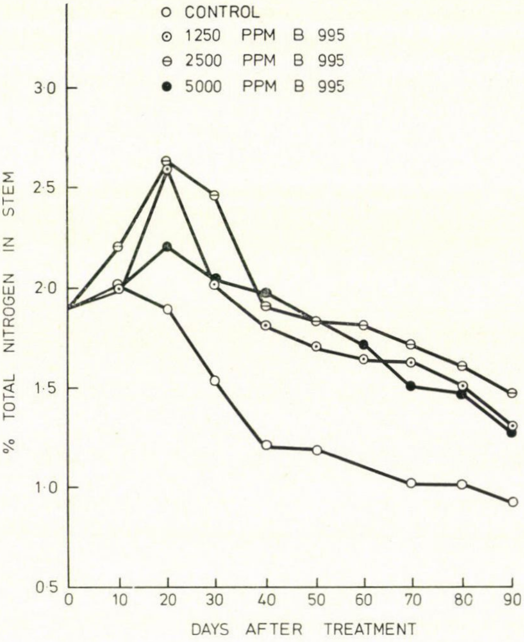

Fig. 6. Response of tobacco plants to foliar sprays of B-995 in nitrogen

The increase in the number of floral buds and size of inflorescence in the present investigation can be supported by the findings of **Stuart** (1962), **Shanks—Link** (1963). They observed that the application of growth retardants, namely phosphon, CCC, and B-995 caused suppression in vegetative growth but definite increase in the number of floral buds and inflorescence size in the case of *Rhododendron*, hydrangea and poinsettia flowering plants. This may be due to the modifying activity in the cambium cells. Further it is possible that the growth retardants directly affect the biochemical processes leading to flower induction. It is quite likely that with the passage of time the growth retardant present in the plant is reduced to such a level as may influence the flower production.

The higher accumulation of ash content, nitrogen, phosphorus, and potassium in B-995 treated plants reported in this investigation also confirms that observed in different species of plants (**Poole** 1965, **Knavel** 1969.) It is possible that since this chemical is considered as antimetabolite the change in stem tissue could alter the transport of nutrients and other growth materials. **Cathey** (1964), **Halevy** (1966) explained that the greater number of palisade

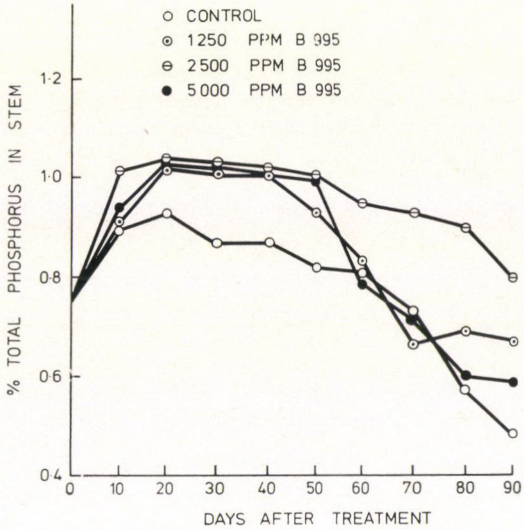

Fig. 7. Response of tobacco plants to foliar sprays of B-995 in phosphorus

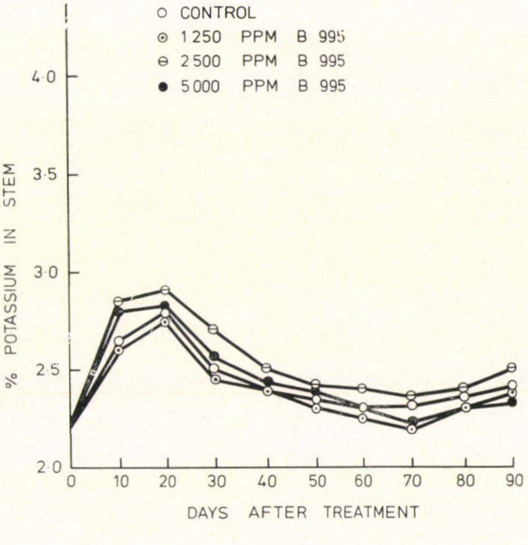

Fig. 8. Response of tobacco plants to foliar sprays of B-995 in potassium

cells and fewer intercellular spaces in the leaves of the treated as compared to the control plants would either tend to decrease the rapid water loss or increase the efficiency of water movement through the leaf tissues. Such an influence on water movement may have extended to the root area which could have resulted in a reduced water uptake as well as transpiration rate and an increased salt accumulation in the plant leaves as suggested by CURTIS (1935). Or its effects may be due to the increased rate of respiration and enzymatic activities as reported by HALEVY (1964) in cucumber seedlings with Amo-1618, and the fact that the salt uptake is directly linked to respiration through the cytochrome system (LÜNDEGÁRDH 1954). So from these observations it is possible to conclude that the higher accumulation of nutrients in this investigation may be attributable to the combinations of the above factors.

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ECOLOGY OF DESERT PLANTS AND OBSERVATIONS ON THEIR SEEDLINGS

IV. Seed germination and seedling growth in *Citrullus* species

The germination of many plants is very poor under laboratory conditions of light and temperature but in nature seeds either appear to germinate quickly or not at all (SEN—CHATTERJI 1968). The hard coat of seeds have been reported to inhibit the germination process in desert seeds. Temperature has been considered to be one of the most crucial factors in the germination regulating mechanism and a special agent in certain conditions for breaking the dormancy of desert and semidesert seeds. HARRINGTON (1916) reported the effect of temperature pretreatment in the germination studies of seeds. Temperature relations of seed germination have also been studied in this laboratory (SEN—CHATTERJI 1966, CHATTERJI—MOHNOT 1967, CHAWAN 1971).

The effect of light in the germination of seeds have been known for many years (CROCKER 1936). The seed germination of different species, under various light conditions have been studied by different workers (EVENARI—NEUMAN 1953, VARSHNEY 1968, SEN 1969, BHANDARI—SEN 1973). It has been shown that the conditions for better germination will also depend on the nature of the soil, its chemical composition, its physiological structure and the depth of the seeds in the soil.

The present work was carried out on the seed germination of *Citrullus colocynthis*, a perennial cucurbit very well adapted to the hot arid conditions of India and *Citrullus lanatus* cultivated or self grown in the rainy season only in the western part of Rajasthan, India.

Studies on seed germination were done in sterilized Petri dishes lined with a single layer of filter paper moistened with distilled water or with test solution. The criterion of germination was the visual detection of the radicle protrusion. Each test was performed in three replications and each Petri dish contained 10 seeds. In case of *C. colocynthis*, judicious moisture was provided as the seeds rot in excess of water or solution as compared to *C. lanatus*. The decoated seeds were sterilized in 10 per cent mercuric chloride solution. The experiments were carried out in continuous light (1000 lux) and total darkness at varying temperatures (23° to 31 °C). Observations were recorded daily, and the final observations and measurements of seedling growth were made after 4 and 6 days in *C. lanatus* and *C. colocynthis*, respectively. The data obtained were statistically analyzed for all experiments.

Both the species possess a very hard seed coat. The removal of the seed coat was done by means of a plier. The decoated seeds obtained in this manner were used for germination experiments.

Intact seeds were exposed to various ranges of temperature from 30° to 90 °C for different durations: 1, 3, 5, 7, 15 and 30 days in a ventilated oven to determine the effect of a dry heat pretreatment. The temperature treatment was provided by placing the decoated seeds in Petri dishes with water (moist heat) and keeping them at different temperatures (20° to 30 °C) in total darkness for the specific period.

Decoated and dark-imbibed seeds were used to observe the effect of different wavelengths of light on germination percentage. Blue, green and red lights were obtained by wrapping the Petri dishes with differently coloured cellophane papers, for total darkness the seeds were kept in light proof cabinets.

The effect of a number of other pretreatments were tried. The effect of a hot water treatment was given by keeping intact seeds in boiling water for the duration of 1 to 15 minutes. The chilling treatment was given by keeping intact seeds in a refrigerator at 0 °C for the duration of 1 to 24 weeks. Intact seeds were kept in absolute alcohol for a duration of 24 to 240 hours for this treatment. The wetting and drying treatment was given for a week to the intact seeds.

To observe the effect of soil types and soil depths, the intact seeds were sown at uniform depths in earthen pots, containing sandy, humus, and clay soils. The seeds were sown in glass jars at different depths and kept in light proof wooden chambers to ensure complete darkness. Water was given when found necessary. Final observations were made after one week of the start of experiments.

a) *Seed coat removal.* The mechanical removal of the seed coat caused 70 to 100 per cent germination in total darkness and only 20 per cent in continuous light. The germination in continuous light was visible by a slight protuberance of the radicle and an expansion of that cotyledon which remained in contact with moisture. This cotyledon enlarged and developed chlorophyll, while the other remained unchanged in size and pigmentation. Localized rotting took place in decoated seeds germinated in continuous light where some damage had been caused by a plier while removing the testa, but this did not happen in those germinated in total darkness.

b) *Dry heat and temperature.* The intact seeds of both *Citrullus* species exhibited no germination after various ranges of dry heat pretreatments. When seed coats of such treated seeds were removed, the germination percentage increased to nearly a hundred per cent. This proved that temperature had no effect on breaking hard seed coat dormancy. With the increasing temperatures (moist heat), the percentage of germination in *C. colocynthis* decreased but the seedling growth was enhanced. Rotting was more frequent in seeds germinated at higher temperatures and the ones germinated showed better growth at those temperatures. In *C. lanatus* the percentage of seed germination also decreased with increasing temperatures (Table 1). The linear growth of the seedlings was accelerated by an increase in temperature.

c) *Light treatment.* The effect of different wavelengths of light on the seed germination and seedling growth of the *Citrullus* species is presented in Tables 2 and 3.

It is evident from Tables 2 and 3 that the seeds of the *Citrullus* species showed a variable germination in different wavelengths of light. Germination was poor in almost all the wavelengths of light at the end of 4 days except in total darkness. A dark incubation period of 24 hours to the above exposed seeds brought about a high percentage of germination in all the wavelengths of light. This showed that the 24 hours of the dark incubation period was insufficient and when the seeds received the optimum dark incubation by another dark incubation for 24 hours germination took place and the effect of different wavelengths of light became secondary. The linear growth of seedlings was maximum in total darkness, followed by red light, while growth remained largely unaffected in others.

d) *Other pretreatments.* Hot water pretreatment, absolute alcohol pretreatment, chilling pretreatment and an alternate wetting and drying pretreatment ot intact seeds showed imbibition, but these treatments failed to bring about the optimum germination percentage. On removal of the testa these treated seeds showed an enhanced germination percentage in total darkness.

Table 1

Effect of different temperatures (moist heat) on the germination percentage of the decoated seeds and the linear growth (in cm) of seedlings in C. colocynthis and C. lanatus at the end of the 6 and 4 days, respectively

Temperature in °C	*C. colocynthis*			*C. lanatus*		
	Germination percentage	Radicle	Hypocotyl	Germination percentage	Radicle	Hypocotyl
22	98.0±5.0	3.4±0.1	0.6±0.1	99.0±6.0	4.0±0.6	1.1±0.4
25	84.0±2.8	3.5±0.2	0.7±0.3	89.6±5.0	5.3±1.0	1.2±0.6
27	78.2±2.0	3.8±0.8	1.5±0.4	76.6±2.8	6.4±1.2	1.8±0.9
29	65.0±3.0	4.9±0.9	1.8±0.3	70.4±3.0	8.4±0.9	2.3±0.8
31	50.0±0.0	6.0±0.2	3.4±0.1	68.3±0.0	10.4±0.8	3.0±0.4
33	42.2±0.0	7.1±0.4	5.8±0.2	60.0±0.0	11.2±0.6	4.6±0.7
35	35.0±3.0	9.5±0.9	6.1±0.1	51.9±3.0	13.2±2.0	5.9±0.4

Table 2

Effect of different wavelengths of light on the germination percentage of decoated seeds and the linear growth (in cm) of seedlings in C. colocynthis

Wavelengths of light	Germination percentage after 4 days	Germination percentage after 7 days*	Growth of seedlings after 7 days	
			Radicle	Hypocotyl
Green	20.0±1.0	80.0±5.0	1.7±0.2	0.3±0.1
Blue	23.3±2.5	83.0±4.0	1.7±0.3	0.2±0.2
Red	36.0±5.1	90.0±6.0	2.3±0.5	0.3±0.0
Total darkness	76.0±1.0	98.0±8.0	4.0±0.1	0.6±0.4
White	25.0±1.0	85.2±2.0	2.0±0.5	0.3±0.1

* 24 hours dark incubation after 4 days exposure to different wavelengths of light

e) *Effect of soil types and sowing depths.* The germination of *C. colocynthis* was found to be the best in sandy soil and that of *C. lanatus* in humus soil. Clayey soil proved unfavourable for germination in both the species (Table 4).

In *C. colocynthis*, the germination percentage was 60 per cent when sown at a 2 cm depth; while it was 80.2 per cent at 3 cm in *C. lanatus* (Table 5).

Germination was visible in glass jars at 2 and 3 cm but the seedlings came out earlier from land 2 cm deep both in *C. colocynthis* and *C. lanatus*.

Seeds of a large number of desert species are reported to germinate abundantly in nature, but ordinarily they failed to do so under laboratory conditions (SEN—CHATTERJI 1968). *C. colocynthis* is an example of the type where germination under laboratory conditions was difficult or extremely poor.

Application of dry heat at different temperatures ranging from 60° to 100 °C caused higher imbibition and germination (SEN—CHATTERJI 1966, CHAWAN 1971). Such a treatment was not found to produce any effect on the intact seeds of any of the *Citrullus* species. In decoated seeds of both the *Citrullus* species, the germination percentage decreased with increasing high temperatures (and increased with falling temperatures). At the same time a high temperature favoured the seedling growth. Sen—Chawan (1970) showed that the higher temperature suppressed the seedling growth of *Astercantha longifolia* both in continuous light and total darkness. Koller *et al.* (1963) reported that naked embryos of *C. colocynthis* germinated at all temperatures between 20° to 35 °C and their growth was found strongly favoured by the high temperature and darkness.

Mayer—Mayber (1963) stated that seeds might be divided into: those germinating only in the dark; germinating only in continuous light; germinating after being given a brief illumination; and those which were indifferent to the presence of absence of light during germination. The seeds of *Citrullus* species fall in a category where a high percentage of germination took place in total darkness. An interruption by a 24 hours total dark period for seeds germinating in light after the initial 24 hours dark incubation, enhanced the germination percentage. This shows that in reality a 48 hours dark period is necessary for the germination of seeds in *Citrullus* species. These findings do not agree with those of Koller *et al.* (1963) who reported that under light and dark conditions embryos germinated within 24 hours. Sen (1968) reported that continuous illumination appeared to hasten seed germination in *Euphorbia caducifolia*. He also observed that the seeds of desert plants like *Calotropis procera* and *Leptadenia pyrotechnica* were not rigid in their requirements of light and temperature for germination (Sen 1969).

Table 3

Effect of different wavelengths of light on the germination percentage of decoated seeds and the linear growth (in cm) of seedlings in C. lanatus

Wavelengths of light	Germination percentage after 4 days	Germination percentage after 7 days*	Growth of seedlings after 7 days	
			Radicle	Hypocotyl
Green	20.6±2.0	85.0±6.0	2.9±0.6	0.6±0.1
Blue	21.6±1.0	85.0±4.6	2.8±0.7	0.7±0.2
Red	23.3±3.0	90.0±4.5	3.8±0.6	0.9±0.2
Total darkness	81.0±3.3	99.0±3.3	6.2±1.2	2.3±0.3
White	18.3±1.3	85.2±1.3	3.2±0.8	0.9±0.2

* 24 hours dark incubation after 4 days exposure to different wavelengths of light

Koller *et al.* (1963) reported the inhibition of germination in seeds of *C. colocynthis* in blue and green lights. In the present study, poor germination as well as seedling growth was observed in blue and green lights. Sen (1969) reported that *Calotropis procera* was not appreciably affected in any light except white. Blue light was conclusively shown to affect seed germination (Evenari *et al.* 1957).

Extremely low or nearly freezing temperatures have not normally been found favourable in enhancing the permeability in most desert seeds like *Cassia auriculata*, *Heliotropium eichwaldi* (Chatterji 1969). A chilling treatment had no effect on germination in both the species of *Citrullus*. Koller *et al.* (1963) reported that soaking in acetone, immersion in boiling water and a dry heat pretreatment failed to improve germination in *C. colocynthis* which observation agreed with the present work. However, with all the above treatments, the embryo viability was not impaired and upon decoating seed germinated very promptly in total darkness.

Table 4

Effect of different soil types on the percentage of seed germination in C. colocynthis C. lanatus at the end of 7 days

Soil types	*C. colocynthis*	*C. lanatus*
Sand	80.0 ± 4.0	85.0 ± 1.2
Humus	68.0 ± 3.0	96.2 ± 2.2
Clay	60.0 ± 5.0	48.0 ± 2.8

Table 5

Effect of sowing at different depths in sandy soil on the percentage of seed germination in C. colocynthis and C. lanatus at the end of 7 days

Depth in cm	*C. colocynthis*	*C. lanatus*
1	50.0 ± 1.2	55.6 ± 1.0
2	65.0 ± 2.4	60.6 ± 4.0
3	60.2 ± 3.2	80.2 ± 2.0
4	50.0 ± 3.4	55.0 ± 3.0
5	25.0 ± 3.0	35.0 ± 3.8

The inhibitory effect of the testa could be a result of its mechanical resistance to embryo expansion, impermeability to water or other substances or the presence of inhibitors (Koller *et al.*, 1963). In the present investigations, it was observed that differently treated seeds and even the untreated ones absorbed water which caused an increase in their weights. Therefore, it appeared that testa did not present a barrier to water absorption (Koller *et al.* 1963).

The difference in the germination percentage of various types of soils, corresponded to the natural habitat of both the *Citrullus* species. A better germination and growth of the seedlings of *C. colocynthis* in sandy soil and that of *C. lanatus* in humus soil was commonly seen. Montasir—Abed El Rahaman (1951) reported that the highest germination percentage in the *Zygophyllum simplex* was obtained at the superficial level of the soil while the rate of percentage decreased to 11 per cent at a 1 cm depth and became poorer still as the depth increased. In the present investigation *C. colocynthis* exhibited better germination at a 2 cm depth while *C. lanatus* at a 3 cm depth.

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STUDIES ON EGYPTIAN BLACK OLIVES I. RAW MATERIALS USED IN THE PICKLING

The olive fruit *(Olea europaea)* is the most important oil crop cultivated in the oases and the rehabilitated areas in Egypt. The olive oil and the pickled fruits play an increasingly important role in the diets of the people in Egypt. The whole fruit may contain 35—70 per cent of oil (dry basis) (Mattil *et al.* 1964). The pulp often contains over 75 per cent (Mattil *et al.* 1964, Cruess 1938, Jacobs 1951). Ripe olives contain 69.60 per cent moisture, 4—4.3 per cent carbohydrates and 3.40 per cent ash (Cruess 1938, Jacobs 1951). Great efforts are being carried on for the breeding of olive varieties that have high oil content and are suitable for pickling. There is no adequate information concerning the chemical structure of the olives in Egypt. The objective of this study was to evaluate the olive varieties produced in Egypt by two methods of cultivation.

Sampling: *Olea europaea* of Sarrana, Negral, Sevillano, Willd longa, Picual and Arbequin varieties were propagated by cutting and grafting in the Waddy El Natroon oasis, Egypt. Olive fruits of Chemlaley Weatecken and Hammaddy varieties grown as grafts at Siwa oasis, Egypts, were used. The fruits were harvested at ripe stage in December 1972.

Analytical methods: The fruits were cut into halves and the seeds were removed then macerated in a Waring blendor and the resulting sample was taken directly for analysis. Samples used for oil determination were prepared by crushing the whole fruits into a mortar similar to the common method of production by which the whole fruits are crushed before pressing. The samples were analyzed for the moisture content at 105 °C for 3 hours, the ash and the oil (as ether extract) using the usual standard methods (A. O. A. C. 1960).

Table 1

The oil content in the whole fruits of the olive varieties cultivated in Egypt (dry basis)

Variety	Method of cultivation	
	Cutting	Grafting
Sarrana	47.32	50.60
Negral	47.50	50.71
Sevillano	46.22	50.19
Villd longa	43.21	46.94
Picual	45.14	49.01
Arbequin	43.32	47.03
Chemlaley	47.20	
Weatecken	39.04	
Hammaddy	34.55	

Acidity was determined by titrating the olive fruits after water extraction by 0.1 N Na OH until PH 8.0 using a Radio pH-meter. The same apparatus was used for the pH value determination. Sugars were identified by the method previously mentioned by SALEM—HEGAZI (1973). The Munson and Walker gravimetric method as described by WINTON—WINTON (1947) was used for the determination of sugars.

The chemical analysis of the olive fruits grown in Egypt is shown in Tables 1 and 2. The oil content in the whole olive is in the range previously mentioned by others (MATTIL *et al.* 1964). Table 1 indicates that the grafting method caused a high oil content in the fruits. Negral, Sarrana and Sevillano olive varieties are the highest from the oil content point of view. Weatecken and Hammaddy have the lowest oil content.

The total solids, pH, acidity, ash and sugar contents of Egyptian olive varieties produced by cutting and grafting cultivation methods are shown in Table 2. The pH and acidity have been of particular interest for pickling purposes. The pH ranged from 5.3 to 6.1 and the acidity per cent (as citric acid) from 0.34 to 1.20. The ash content ranged from 3.83 to 5.53 per cent. Sarrana had the highest value while Negral had the lowest. Sugars are an important quality attribute for the pickling of the olive fruits. However, sugars are responsible for lactic acid fermentation during pickling. The total sugar per cent ranged from 0.67 to 5.84. Chemlaley had the highest, while there were 0.67 per cent in the Arbequin. Weatecken and Hammaddy

Table 2

Chemical characteristics of olive varieties produced by cutting and grafting methods of cultivation and grown in Egypt (dry basis)

Variety	Total solids %	Ph	Total acidity % (as citric acid)	Sugars			
				Ash %	Fructose and glucose	Sucrose	Total
1-Cutting method							
Sarrana	35.62	5.3	1.13	5.53	2.29	0.00	2.29
Negral	34.53	5.5	0.86	3.83	3.38	0.86	4.24
Sevillano	33.22	—	—	4.94	1.08	1.39	2.47
Villd longa	32.65	5.5	0.96	4.59	1.16	0.00	1.16
Picual	33.22	5.9	0.45	4.99	2.69	0.00	2.69
Arequin	34.15	5.8	0.74	4.93	1.29	0.00	1.29
Chemlaley	39.88	5.4	0.48	4.50	5.84	0.00	5.84
Weatecken	35.88	5.4	0.59	4.49	3.86	0.00	3.86
Hammaddy	35.80	5.3	0.65	4.70	3.72	0.00	3.72
2-Grafting method							
Sarrana	34.92	5.8	0.49	4.84	1.80	0.00	1.88
Negral	35.12	5.9	0.62	4.01	1.68	0.00	1.68
Sevillano	34.44	5.7	0.69	4.17	1.12	0.00	1.12
Villd longa	33.11	5.3	1.20	4.71	0.97	0.00	0.97
Picual	34.52	6.0	0.49	5.34	1.16	0.00	1.16
Arbequin	33.95	6.1	0.34	3.88	0.67	0.00	0.67

Cutting showed a decrease in pH and acidity and an increase in sugars and ash contents

varieties had 3.86 and 3.72 per cent. Sugars were identified as fructose, glucose and sucrose. Sucrose was identified only in Sevillano and Negral, meanwhile other varieties contained fructose and glucose.

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N BALANCE IN FLOODED RICE CULTURE IN RELATION TO METHODS OF APPLICATION

Among plant nutrients N is usually the most limiting for higher rice yield in India. The current shortage of N fertilizers and high cost calls for its efficient use. This is important from the point of view of possible nitrate pollution as well, particularly at high levels of application. In flooded rice culture, applied N is subjected to gaseous loss through volatilization and denitrification (ABICHANDANI—PATNAIK 1958, GUPTA 1955), and leaching in light-textured soils (PANDE—ADAK 1971). In addition, a considerable amount may be tied up in the soil through biological immobilization particularly when straw is incorporated, and it is not certain how much of this is available to meet the current demand of the growing crop. It is, therefore, not surprising that in field experiments usually only 28 to 34 per cent of the applied N is recovered in plant uptake (RAJENDRA PRASAD *et al.* 1971). Split application, deep placement, slow-release source, and intrification-inhibitors have been tried to achieve greater efficiency of applied N with varying degree of success. This paper describes an attempt to draw a N balance sheet from a pot-culture experiment in which urea was applied through soil balls with and without deep incorporation of rice straw and through soil plus straw balls.

Four kg of sandy loam, surface soil (0—15 cm) from the Institute farm (pH 6.8, total N 0.018%, organic C 0. 193%, C. E. C. 5 m.e./100 g, and exchangeable NH_4—N 5 ppm) was placed in tall pots with drainage line underneath. Pebbles-followed by washed river sand were placed between the soil and the drainage line to facilitate leachate collection. The potted soil was mixed with 1.1 g of single superphosphate and 0.3 g of KCl to give 100 kg per ha P_2O_5 and K_2O. Each urea-straw-soil ball was composed of 0.1 g urea in solution form, 1.35 g ground rice straw and 12.0 g air dry soil. The soil acted to give a cohesive ball. The composition of urea-soil balls was similar except that straw was not included. To check the loss of urea solution, the components were mixed over a polythene sheet and then shaped into balls without direct contact with the hand. The balls were air-dried in shade under a fan. Each ball was equivalent to 25 kg N per ha without considering the 0.4 per cent N in the straw. For split applications, the balls were prepared a few days before incorporation.

The soil was puddled by hand and N was applied as follows: — (1) basal application of urea — soil balls, (2) split application of urea-soil balls, (3) basal application of urea-straw-soil balls, (4) basal application of urea-soil balls with a 3 cm soil plus straw (500 g + 8.1 g) layer at 20 cm depth, (5) split application of urea-soil balls with soil plus straw layer as in Treatment 4, and (6) split application of urea granules, the basal dose being applied on soil surface followed by light puddling up to 2 cm depth and the rest being top-dressed on flood water. The balls were pushed to a depth of 8 cm in both basal and split applications. In basal application 6 balls (150 kg N/ha) were applied a day before transplanting. In split treatment 3 balls (75 kg N/ha) were applied basally followed by 2 and 1 (50 and 25 kg N/ha) at maximum tillering and flowering stages, respectively. In Treatments 3, 4 and 5 the total quantity of straw per pot was kept uniformly at 2 per cent level (W/W). Each treatment was replicated 6 times.

Rice seedlings (*Oryza sativa* L. Ratna) were transplanted at the rate of 3 per pot. Soon after seedling establishment the pots were flooded to a depth of 8 cm, the drainage line was opened and connected to individual bottles for leachate collection. Fifty ml of leachate was collected per pot per day during the entire growth period, approximating a vertical percolation loss of 3 mm per day on soil weight basis. The leachates were acidified and stored in a refrigerator to prevent fungal growth. Seven days' collections from individual pots were pooled and 2 composite samples made by mixing the leachates from 3 replicates each of a treatment. The duplicate samples were analyzed for mineral $(NH_4 + NO_3)$—N by the micro-distillation procedure (Bremner 1965). The cumulative loss of mineral-N through leaching was expressed as mg per pot. The N input through irrigation water was calculated from the volume used daily to maintain the flood level and the concentration (2.5 ppm). The growth of blue-green algae was severely affected, probably due to unusually low air temperature during the winter season, and therefore the N contribution from this source was not considered.

Table 1

N balance sheet

Treatment	Total N input mg/pot a	N out go through		Organic mineralizable N in the mg/pot	N recowered in the crop	Total N input accounted for mg/pot	Not accounted N, % of total input
		crop removal mg/pot b	leaching mg/pot		% of total N input		
1. Urea+soil balls, basal application (150—0—0)	333.0	233.5	55.0	24.5	70.12	313.0	6.1
2. Urea+soil balls, split application (75—50—25)	333.0	187.5	45.5	30.6	56.30	263.6	20.9
3. Urea+straw balls, basal application (150—0—0)	365.4	115.8	42.4	136.4	31.69	294.6	19.4
4. Urea+soil balls, basal application with straw layer below (150—0—0)	365.4	202.0	38.4	115.6	55.28	356.0	2.6
5. Urea+soil balls, split application with straw layer below (75—50—25)	365.4	203.3	25.2	86.7	55.63	315.2	13.8
6. Urea granules, split application (75—50—25)	333.0	121.2	50.6	55.0	36.39	206.8	37.9

a — Total of 270, and 20 mg N/pot from urea, irrigation water and soil (exchangeable + mineralizable) respectively. Treatments 3, 4 and 5 additionally contained 32.4 mg N/pot through straw

b — C.D. 5%, 29.58

The shoot portion of one plant per pot each at maximum tillering, flowering and maturity was harvested, oven dried (80 °C), and analyzed for total N following Kjeldahl digestion and Nesselerization. The grain samples were analyzed separately. From these analyses, the N uptake at different stages and the total recovery in the crop was calculated. Immediately after final harvest, the entire pot soil was evacuated taking precaution to leave behind the sand and pebble layer and then air-dried. The plant roots were removed by sieving and hand picking. Mineralizable N in the soil was determined by the anaerobic incubation procedure (Waring—Bremner 1964). The nitrate-, exchangeable-, and clay fixed-N was only in traces. The mineralizable portion represented the bulk of residual, organic-N and the "not-accountable"-N the gaseous loss through denitrification/volatilization, in accordance with the general practice. No direct measurement of the latter was attempted.

The N balance sheet (Table 1) showed that 70 per cent of the total N input was recovered in the crop on basal application of urea-soil balls at 8 cm depth. The low residual, organic-N in the soil and not-accountable fraction indicated negligible immobilization and gaseous loss through volatilization and/or denitrification. This method of application, therefore, resulted in efficient N uptake, particularly during the tillering stage (Table 2), and significantly higher grain and straw yields (Table 3). Split application of urea granules, however, resulted in a significantly lower recovery of N by the crop and poor straw and grain yield, probably because of about 38 per cent loss of N through gaseous products. But when the split application was made using urea-soil balls, the N recovery and straw and grain yield improved. The gaseous and leaching losses were also reduced to nearly 21 and 13 per cent, respectively. Placement of split application in the reduced zone is known to decrease gaseous losses. However N fertilizers are usually broadcast over flood water, leading to heavy losses. The use of urea-soil balls, which can be easily pushed down, may prove quite successful in areas where the fields cannot be drained before top-dressing on account of either topographical reasons, or uncertain availability of water for reflooding.

Table 2

Concentration and total uptake of N (oven dry basis)

Treatment	Tillering stage		Flowering stage		Final harvest			
					Straw		Grain	
	Conc. mg/g	Total uptake mg/pot	Conc. mg/g	Total uptake mg/pot	Conc. mg/g	Total uptake mg/pot	Conc. mg/g	Total uptake mg/pot
1. Urae+soil balls, basal application (150—0—0)	18.00	61.44	9.66	57.07	5.61	46.89	6.31	68.08
2. Urea+soil balls split application (75—50—25)	12.84	29.45	14.36	56.24	5.61	34.66	7.86	67.16
3. Urea+soil+straw balls, basal application (150—0—0)	15.34	36.12	11.51	36.85	4.67	16.87	7.94	25.93
4. Urea+soil balls, basal application with straw+soil layer below (150—0—0)	16.03	55.48	12.40	68.38	4.07	22.08	8.16	56.01
5 Urea+soil balls, split application with straw+soil layer below (75—50—25)	15.55	30.75	20.63	73.96	5.88	36.71	8.64	61.85
6. Urea granules, split application (75—50—25)	14.92	14.80	19.75	46.80	6.38	25.01	9.09	34.63
C.D. 5%	4.36	19.19	2.30	17.05	1.10	7.10	1.98	18.41

Basal application of urea-straw-soil balls caused low crop recovery of N (about 31.7 per cent) and straw and grain yield at harvest, which were not significantly different from those with a split application of urea granules. However, the former method helped to conserve N by reducing the leaching and gaseous losses, and by augmenting the organic pool which could be tapped by a succeeding crop. It may also reduce the denitrification loss that occurs on alternate flooding and drying of rice fields. Despite, these benefits, immobilization, which accounted for 37.3 per cent of the total N input, appeared to hinder N uptake and reduce yield of currently growing crop. BROADBENT—NAKASHIMA (1970) also observed extensive immobilization of N^{15}-labelled ammonium sulphate in a laboratory incubated, flooded soil containing 1 per cent barley straw. On the other hand, WILLIAMS *et al.* (1968) did not find evidence of immobilization that could limit rice yield in a flooded, field culture when rice straw (0.45 per cent N) supplemented with urea was incorporated.

Deep incorporation of straw (Treatments 4 and 5) mitigated, to some extent, the disadvantage of placing it in the root zone (Treatment 3). Lower immobilization appeared to lead to significantly greater N uptake and higher straw and grain yield; the leaching and gaseous losses were also reduced. The comparatively low fraction of applied N which could escape plant uptake probably resulted in smaller leaching loss and immobilization. BARTHOLOMEW (1965) has drawn attention to the importance of available N and the position of plant residues in the soil in connection with immobilization.

Table 3

Straw and grain yield (g/pot) oven dry

Treatment	Straw yield at			Grain yield
	Maximum tillering	Flowering	Maturity	
1. Urea+soil balls, basal application (150—0—0)	3.40	5.75	8.42	10.77
2. Urea+soil balls, split application (75—50—25)	2.28	3.93	6.28	8.35
3. Urea+soil+straw balls, basal application (150—0—0)	2.22	3.21	3.58	3.40
4. Urea+soil balls, basal application with straw+soil layer below (150—0—0)	3.48	5.50	5.40	7.07
5. Urea+soil balls, split application with straw+soil layer below (75—50—25)	1.97	3.60	6.43	7.01
6. Urea granules, split application (75—50—25)	1.00	2.36	3.94	3.80
C.D. 5%	0.75	1.02	1.21	1.44

It is interesting to evaluate the various methods of application in terms of N leakage from flooded rice culture (Table 1). The loss of N through the soil profile, which might pose a threat to the quality of underground or surface waters specially at high rates of fertilization, may be minimized by using straw. Deep incorporation of straw followed by split application of urea-soil balls reduced the leaching loss over 50 per cent of that observed on comparable application of urea granules or urea-soil balls without straw. The interference in N uptake through immobilization was not as severe as that when the straw was placed in the root zone. This method, therefore, afforded the best compromise between the control of leaching losses through biological immobilization and grain yield. The leakage to atmosphere via gaseous products is important to the overall efficiency of applied N. The best crop recovery of N and grain yield with the minimum possible denitrification/volatilization loss was obtained when urea-soil balls were applied basally at the depth of 8 cm.

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IRRIGATION REQUIREMENTS OF MAIZE IN A TROPICAL ENVIRONMENT

Zea mays (maize) is a minor crop in the Sudan, mainly produced by rain. *Sorghum vulgare* (dura), *Pennisetum typhoieum* (bulrush millet) and *Triticum vulgare* (wheat) are the main cereal crops. The consumption of wheat is progressively increasing and the country, so far has managed to produce about half its requirements. The idea of mixing wheat flour with that of a locally more available cereal for making bread has been accepted with the advantage of reducing imports, especially when prices of imported wheat increased sharply. Although the flour of wheat and sorghum have been mixed in certain proportions and marketable bread was made in spite of the inferior baking quality of sorghum, maize appears to be a more attractive substitute to mix with wheat, its yellow pigment being more similar to that of wheat and the taste quality is probably better than that of sorghum. In addition to the value of maize for human consumption, the grain is, of course, well-known as a rich feed for animals including poultry and the local and world market have a strong demand for it.

Since the yields of rain-fed maize in the Sudan are unreliable and poor (around 750 kg/ha), the need was strongly felt to carry out some experimental work to determine the yield potential under different levels of irrigation and to assess the efficiency of water use of this crop within the range of soil moisture where economic production is likely to be recommended.

Two experiments were carried out between August 1971 and March 1972 in the fields of the Faculty of Agriculture, near Khartoum. The experimental area of the first experiment was under pulses preceded by cotton, the site of the second experiment was previously under beans preceded by wheat. The land was ploughed, disced and levelled. The experimental plots were 4 × 4 m, the borders of which were excavated to the depth of 90 cm in which overlapping, galvanized iron sheets were installed and buried to ground level. At the surface the plots were separated by earthen embankments to about 60 cm high and 70 cm wide. The experimental

Table 1

Water received during the season

	First experiment				Second experiment		
Treatment	before treatments plus rain mm	after treatment rain mm	after treatment irrigation mm	Total mm	before treatments mm	after treatments mm	Total mm
A	253	17	360	630	150	560	710
B	253	17	220	490	150	385	535
C	253	17	140	410	150	280	430
D	253	17	82	352	150	246	396

N.B. The rainy season ended before the beginning of the second experiment

Table 2

Means of some attributes of growth at harvest I.
(N.B. in Tables 2 to 4 each mean was based on four samples (one per plot). Crop height was the mean of 12 plants measured in the field)

	First experiment				Second experiment			
Treatment	Crop height cm	Dry weight of leaves g	Dry weight of stems g	Leaf area index	Crop height cm	Dry weight of leaves g	Dry weight of stems g	Leaf area index
A	160.0	45.6	83.9	6.42	171.9	48.0	68.8	6.24
B	151.8	42.5	71.0	5.80	173.5	42.8	63.5	6.25
C	141.5	43.0	60.6	6.32	156.1	45.6	70.1	5.62
D	126.2	45.4	62.1	6.81	144.4	41.6	55.0	5.26
S.E.	4.1	1.6	4.4	0.24	3.8	1.8	4.4	0.49

site was surrounded by an adequate guard area of maize. Irrigation water was highly controlled by a system of metal pipes, control vales, and an adjustable pressurized rubber hose. Water was supplied by a pump of known discharge, withdrawing water from a reservoir of maintained suction-head. The predetermined quantity of irrigation water was directly discharged at the plot as flood irrigation.

A local variety of maize, "Hudaiba 113", recommended by the Agricultural Research Station for the Northern Sudan, was chosen for the present studies. The sowing date of the first experiment was on 11. 8. 71, about the middle of the short monsoon season, during which maize is grown traditionally. The crop of the second experiment was sown on 16. 11. 71, by the onset of the cool dry season which also fitted within the recommended sowing date for maize under irrigation in Northern Sudan (IMMAM 1969). Five seeds per hill were sown by hand at 20 cm spacing and 50 cm between the rows. The plants were thinned to two per hill 20 days later and again thinned to one seven days thereafter to achieve uniformity of stand of 100,000 plants per ha. At sowing ammonium sulphate was broadcast at the rate of 375 kg per ha. The

Table 3

Means of some attributes of growth at harvest II

	First experiment					Second experiment				
Treatment	Dry height cm	Dry weight of leaves g	Dry weight of stems g	Dry* weight of ears	Leaf area index	Dry height cm	Dry weight of leaves g	Dry weight of stems g	Dry* weight of ears g	Leaf area index
A	201.3	51.1	104.8	103.8	6.66	198.2	67.4	123.0	12.3	9.14
B	196.5	71.0	92.5	61.8	8.39	190.2	64.0	112.5	14.8	7.25
C	166.3	67.0	98.0	33.8	8.49	172.8	69.0	103.0	13.8	8.04
D	162.5	60.0	115.5	81.0	7.01	148.6	53.0	99.0	7.0	7.07
S.E.	5.1	4.4	7.4	13.2	0.48	4.9	3.8	5.7	2.0	0.43

*Rachis + developing grain

Table 4

Means of some attributes of growth at harvest III

	First experiment					Second experiment				
Treatment	Crop height cm	Dry weight of leaves g	Dry weight of stems g	Dry* weight of ears g	Leaf area index	Crop height cm	Dry weight of leaves g	Dry weight of stems g	Dry* weight of ears g	Leaf area index
A	206	74.7	130	157	8.17	232	50.0	87.0	41.5	6.05
B	185	52.7	115	79	6.23	221	50.5	99.5	58.3	5.54
C	174	50.0	92	43	6.06	204	43.4	82.8	32.0	4.90
D	165	55.5	131	56	5.52	191	44.1	70.0	15.6	5.48
S.E.	4.4	3.3	8.9	17.9	0.33	4.8	2.5	5.8	5.0	0.32

* Rachis + developing grain

plots were hand weeded twice during the first month after sowing and then there was no need for weeding.

Four water treatments were included in the study as follows:

Treatment A received irrigation every five days at 40 mm per irrigation; B every 10 days at 55 mm; C every 15 days at 70 mm; D every 20 days at 82 mm. Each treatment was replicated four times in a randomized block design. Soil moisture was sampled gravimetrically at the end of each irrigation interval, at dephts of 15, 30, 45, 60, 75 and 90 cm; in addition periodic measurements of soil moisture were made, using the neutron soil moisture meter at depths from 10 to 120 cm. The irrigation treatments were introduced 30 and 33 days after sowing for the two experiments respectively, i.e. during the vegetative period when the plants had an average height of about 60 cm. The water received by the crop from rain or irrigation

Table 5

Components of final yield of the first experiment (means were based on four plots/treatment)

Treatment	Number of ears per plot	Grain yield per plot kg	Grain yield per ha kg	Litre weight g	Weight of air dry shoots per plot* kg
A	123	4.823	3014	760.5	16.308
B	92	1.921	1200	705.5	16.251
C	72	1.335	834	689.0	15.346
D	65	1.016	635	676.0	14.270
S.E.	9.2	0.387		12.2	0.507

* "Weight of air dry shoots (moisture content about 11%) included all the aerial parts except the grain

Moisture content for the grain was about 10%

Table 6(a)

Components of yield of the second experiment (means were based on four plots/treatment)

Treatment	Number of ears per plot	Grain yield per plot kg	Grain yield per ha kg	Litre weight g	Weight of air dry shoots* kg
A	178	10.489	6556	797.7	20.030
B	145	7.125	4453	738.4	16.516
C	126	5.581	3488	721.5	14.944
D	122	3.977	2486	708.2	14.505
S.E.	6.5	0.651		12.4	1.792

* Weights of air dry shoots (about 11% moisture) included all the aerial parts except the grain. Moisture content of the grain was about 10%

before and after the treatments were introduced in the two experiments is summarized in Table 1 and Table 8b.

In both experiments the growth and development of the crop was followed during the growing season by taking three harvests, in the first experiment the first harvest was taken on 28. 9. 71 (18 days after applying Treatment A), followed by two more at 10 day intervals. In the second experiment the first harvest was on 12. 1. 72 (24 days after applying Treatment A) and the other two were at 10 day intervals (Tables 2, 3 and 4). Tables 5 and 6(a) summarize the components of final yield for the two experiments respectively. Table 6(b) gives a summary of gross returns from the different irrigation policies. Table 7 presents values of the efficiency of water use for the two experiments.

Table 6(b)

A comparison of extra returns from one hectare (in Sudanese pounds) due to extra irrigation above treatment D*

Treatment	D
A	50.00
B	25.44
C	16.64

* One Sudanese pound approximately = 1.2 English pounds. Gross returns were calculated on the basis of the difference between the gross income from the value of extra grain and the cost of extra irrigation, accepting the present irrigation water rates of 7.5 pounds per irrigation of 75 mm per ha and a gross income to the grower of only 20 pounds per ton

Table 7

The effect of irrigation practice on irrigation afficiency

	First experiment				Second experiment			
Treatment	Days to maturity	Average water use per day mm	Consumptive use*	Water use efficiency **	Days to maturity	Average water use per day mm	Consumptive use*	Water use efficiency**
A	85	7.4	477	2.090	111	6.4	372	1.083
B	80	6.1	431	4.081	111	4.8	362	1.201
C	72	5.7	393	4.914	106	4.1	335	1.233
D	72	4.9	368	5.543	106	3.7	343	1.593

* Consumptive use (transpiration ratio) = grammes of water used in the whole season per gramme dry matter produced (grain + aerial parts)

** Water use efficiency = volume of water (in cubic meters) used to produce one kg of grain

The influence of the irrigation treatments on the measured parameters of growth showed a trend of decreasing total dry matter (leaves, stems and ears) due to the combined effects of the irrigation interval and decreasing the quantity of water. Significant differences were detected between some of the growth parameters in the three harvests. However, the partitioning of dry matter into leaves, and stems in Harvest II and III was influenced by the extent of reproductive development, in terms of dry weight of ears, which may be accompanied by drying or senescence of older leaves.

The yield of grain declined very sharply with decreasing total amount of irrigation water in the first experiment, but the decrease in the second experiment was more gradual. In both experiments differences in yield were significant at 5% as a minimum and differences up to the 0.1% level were also detected in both seasons. The irrigation treatments in the second experiment resulted in markedly higher yields than in the first experiment, but the maturation period was longer and the total volume of water applied was bigger in the second experiment. On the other hand, the average water use per day, and consumptive use were higher in the first experiment, thus the efficiency of water use was also low (higher numerical values). This

Table 8(a)

Mean daily 'emperature (weekly average) and total weekly pan evaporation (class A pan)

	First experiment (from 11 8 1971 to 2 11 1971)		Second experiment (from 16 11 1971 to 6 3 1972)	
Weeks	Mean daily temperature (weekly average) °C	Total weekly pan evaporation mm	Mean daily temperature (weekly average) °C	Total weekly pan evaporation mm
1	30	87	27	83
2	29	82	28	75
3	31	104	27	72
4	32	108	26	75
5	30	84	19	70
6	30	86	17	62
7	32	97	19	67
8	32	102	22	70
9	31	91	21	70
10	31	95	23	67
11	29	95	24	79
12	29	102	19	73
13	End of experiment.		22	77
14			23	86
15			26	82
16			28	106

was due to the more favourable environmental conditions for growth during the second experiment, namely warm days and cool night temperatures during November and early March, compared to the higher temperatures in the period August—October during which the first experiment was conducted. Although significant differences in the air dry weights of shoots (excluding grain) between the different treatments in the two growing seasons were detected, yet the magnitude of such differences was substantially smaller than the differences in the grain yield. This suggests that maize as green fodder can be produced successfully in both seasons in the Khartoum region. Leaf area index and grain yield in the second experiment compare favourably with experimental results for three hybrid varieties of maize grown in South-east England, with supplementary irrigation during tasselling and the early grain fill period (ADELANA—MILBOURN 1972). The date of grain harvest was different for plots having different irrigation regimes in both seasons; Treatment C and D in the second season were ready to harvest only five days earlier than A and B. In the first experiment the differences in the maturation period were more pronounced, reaching 13 days between A and D; a similar response was shown for wheat (EL NADI 1969).

Soil moisture determinations at the end of both experiments showed that moisture storage up to the depth of 90 cm was only 30 to 40 mm in the different treatments. Earlier determinations using the neutron soil moisture probe showed little or no changes in moisture

Table 8(b)

Rainfall during the experimental period
(N.B. The first experiment was carried out during the rainy season which ended before the beginning of the second experiment)

Date 1971	Rainfall mm
1.7	0.2
2.7	0.9
21.7	13.6
27.7	24.9
9.8	4.1
11.8	3.6
15.8	1.3
18.8	0.1
20.8	25.7
10.9	12.4
13.9	5.4
17.9	11.6
Total in season	103.8

contents below 90 cm due to the limited infiltration of the heavy, montmorillonitic clay. Therefore, ignoring the small amounts of moisture storage, practically all the applied water can be considered to be lost by evapotranspiration. The average amount of water used per day in both experiments declined steadily with decreasing the total quantity of irrigation in the season and this corresponded to a decrease in consumptive use in seven occasions out of eight. However, when the efficiency of water use was judged by the volume of water required to produce a unit weight of grain, the relationship was reversed and higher water inputs proved to be more efficient viz. the higher the water input the less was the water utilized to produce one kg of grain, and this was valid, without exception, in both seasons. These facts should lead to a revision of the interpretation of consumption use values, since it has often been wrongly accepted that lower consumption use values indicated a measure of water economy, ignoring the economic yield. This is clearly illustrated by the fact that the grain development can be greatly retarded due to water deficiency even if the vegetative development was fairly good during the season.

Table 6(b) emphasizes the extra benefits from light irrigations (at 40 mm) applied every five days. However, should this practice be criticized on the grounds that it requires extra attention by the grower and creates competition for water supply to other crops in the field, strong reasons still hold for a recommendation of irrigation every 10 days (at 55 mm) and the profitability of this practice is undeniable, even if the growers obtain only 50% of the experimental yields.

There is a great potential of maize under irrigation in Northern Sudan, especially when hybrid varieties are used commercially and the present work shows that the high yielding capacity of a locally selected variety when sown in the right time, can produce adequate yields compared with other maize producing countries (COMMONWEALTH SECRETARIAT 1973). Taking two neighbouring countries as an example, the national average yield in Kenya is 1.25 tons/ha and under favourable experimental conditions between 5.24 and 6.18 tons/ha (ALLAN 1971). In the Egyptian Arab Republic the most recent national average under irrigation is 4.04 tons/ha (COMMONWEALTH SECRETARIAT 1973).

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CONTRIBUTION OF SYMBIOSIS TO THE NITROGEN NEEDS OF SOYBEAN (GLYCINE MAX L. MERR.)

Inoculated and uninoculated nodulating and non-nodulating isolines of "Clark" and "Harosoy" were raised at varying levels of N at the Crop Research Centre, Pantnagar. The isolines of 'Clark' were grown in three consecutive season; i.e. the monsoon season of 1970 (Trial I) and the summer seasons of 1971 and 1972 (Trial II and Trial III), whereas those of 'Harosoy' were grown only in the summer seasons of 1971 and 1972 (Trials II and III). There were 5 N levels in Trial I (0, 25, 50, 100 and 200 kg N/ha), whereas one more level, i.e. 300 kg were added in Trials II and III; modification in N levels being based on the results obtained in Trial I. Besides, 43.6 kg P as single superphosphate and 40 kg K as muriate of potash were uniformly mixed in the soil before sowing in all the trials.

Nodulation studies were made at 35 and 65 days after planting. Five plants were sampled on each date by removing a ball of the soil covering the major root mass. The roots were washed to recover the nodules which were separated from the root, counted and weighed after drying.

For N uptake studies 5 plants were sampled at fortnightly intervals starting from 20 days after planting up to 80 days. Thereafter, sampling was done only at harvest, irrespective of time taken for maturity under different treatments. The plants were separated into different plant parts viz. stem, leaves, petioles, flowers, pods, pod-husks and grains as per stage of growth

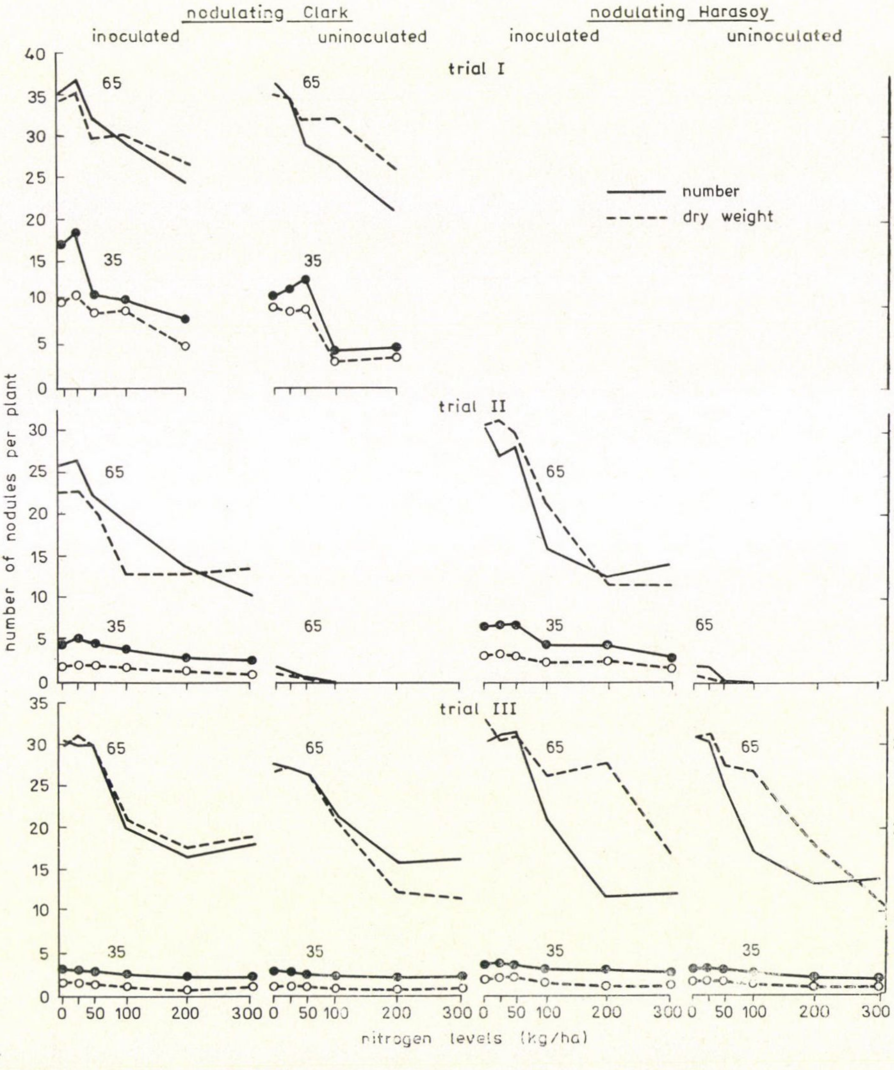

Fig. 1. Number and dry weight of the nodules per plant at 35 and 65 days after sowing as affected by inoculation and nitrogen fertilization. Figure on the curve indicates days after planting when study was made

and oven-dried at 100 °C for dry matter accumulation studies. The different plant parts thus obtained, were analyzed for their total N content using a micro-kjeldahl method Total N accumulation per plant was determined by summing up the N accumulation in different plant parts. Nitrogen uptake per hectare was computed by multiplying total N accumulation per plant with final plant stand.

The contribution of symbiosis was calculated by subtracting the N uptake of non-nodulating isolines from that of nodulating isolines at harvest. This was computed both for inoculated and uninoculated conditions so as to find out the effect of N fertilization on symbiosis as

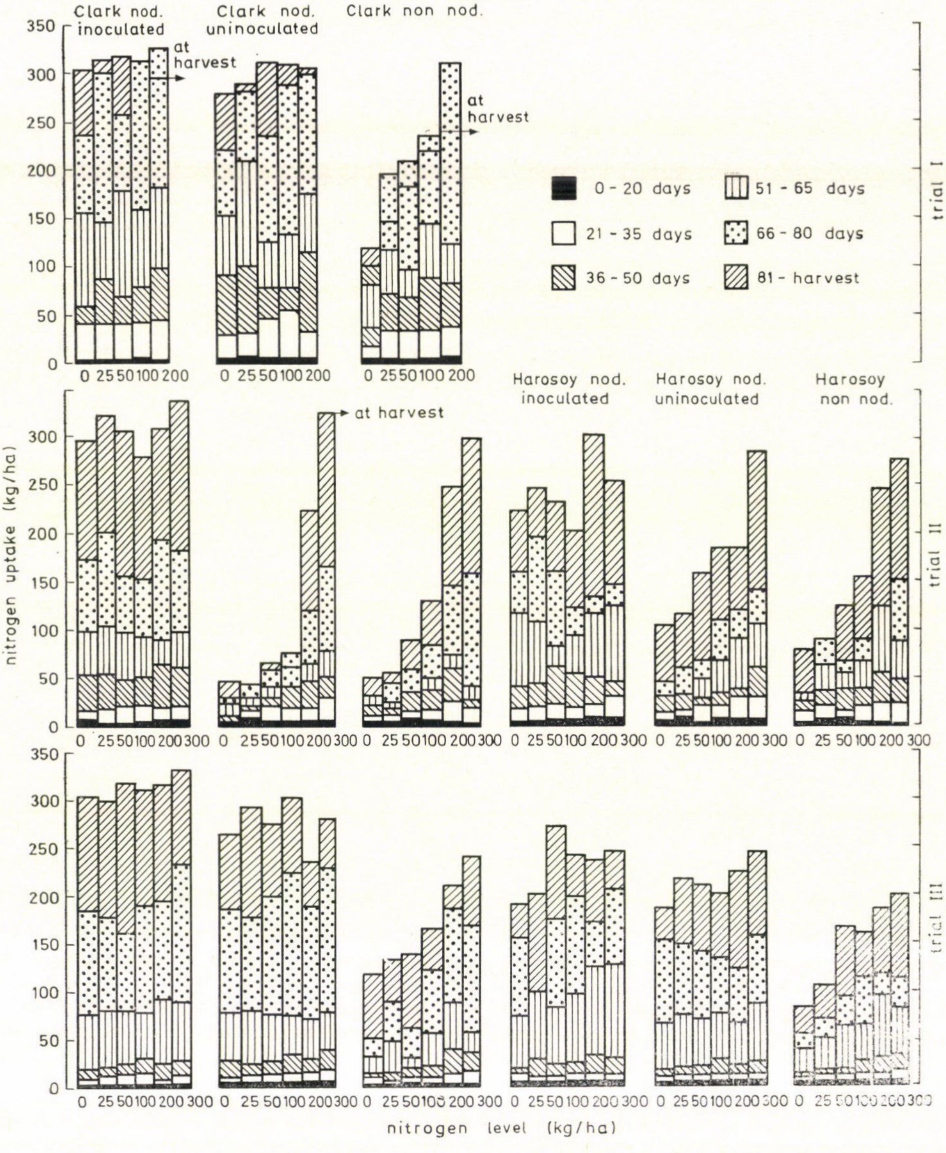

Fig. 2. Nitrogen uptake (kg/ha) at various stages of growth as affected by nitrogen levels in different trials

a function of introduced as well as local rhizobial population. Per cent contribution of symbiosis was calculated by using the equation:

$$\% \text{ contribution} = \left(\frac{Y_1 - Y_2}{Y_1}\right)(100, \text{ where } Y_1 \text{ stands}$$

for N yield of nodulating isolines and Y_2 for N yield of non-nodulating isolines.

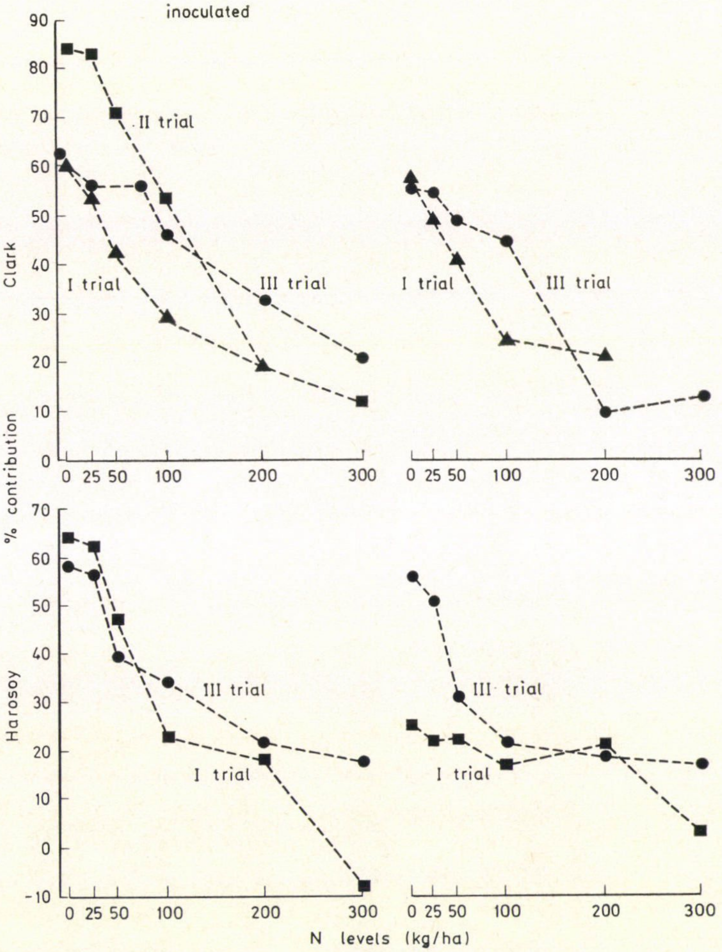

Fig. 3. Contribution of symbiosis to total nitrogen uptake as affected by N levels

Fertilizer N equivalent to symbiotically fixed N was calculated by using the following equation (WEBER 1966):

$$\text{Fertilizer N equivalent} : \frac{\text{Symbioticallv fixed N}}{\text{b}},$$

where *b* stands for the increase in N uptake of non-nodulating isoline for every kg of applied N, the value of which was determined by regression analysis.

Nodulation studies. Inoculated and uninoculated plants of Clark in Trials I and III and of Harosoy in Trial III showed similar degrees of nodulation, although there was a trend for nodulation to be slightly higher under inoculated conditions (Fig. 1). The latter was particularly true 35 days after sowing in Trial III. However, in Trial II, there was considerable difference between inoculated and uninoculated plants in the nodulation. At 35 days after sowing, the uninoculated plants had no nodulation, whereas the inoculated plants exhibited considerable

Table 1

Nitrogen uptake contributed by symbiosis at harvest as affected by nitrogen levels

N levels (kg/ha)	Contribution of symbiosis (kg/ha and %)		
	Inoculated		
	Trial I	Trial II	Trial III
Clark			
0	182.6 (60.8)	147.2 (83.6)	182.8 (60.9)
25	162.4 (53.9)	267.4 (83.1)	163.7 (55.3)
50	131.8 (41.8)	216.2 (71.2)	176.2 (55.9)
100	88.8 (28.8)	150.2 (54.0)	143.0 (46.4)
200	54.5 (18.5)	59.1 (19.2)	102.7 (32.8)
300	—	40.8 (12.0)	62.9 (20.9)
Harosoy			
0	—	143.8 (64.4)	108.9 (57.7)
25	—	153.4 (62.5)	136.9 (56.5)
50	—	109.5 (46.6)	105.2 (38.8)
100	—	47.4 (23.2)	80.7 (33.7)
200	—	54.2 (18.0)	51.1 (21.6)
300	—	—21.6 (—8.4	44.4 (18.0)

N levels (kg/ha)	Uninoculated		
	Trial I	Trial II	Trial III
Clark			
0	161.2 (57.8)	—2.6 (—5.7)	147.4 (55.6)
25	139.4 (48.6)	—10.4 (—23.8)	163.5 (55.3)
50	128.2 (41.1)	—22.9 (—35.6)	135.7 (49.3)
100	86.6 (24.0)	—66.0(—106.9)	135.2 (45.0)
200	65.1 (21.3)	—23.3 (—10.3)	23.2 (9.9)
300	—	28.8 (21.8)	37.3 (13.3)
Harosoy			
0	—	26.1 (24.7)	105.8 (56.4)
25	—	26.9 (22.6)	110.3 (51.1)
50	—	36.2 (22.4)	64.6 (30.7)
100	—	30.0 (16.0)	42.9 (21.3)
200	—	39.5 (21.2)	42.3 (18.6)
300	—	9.1 (3.2)	41.6 (17.1)

Bracketed figures are percentages

nodulation. At 65 days after sowing some nodulation was observed in an uninoculated condition. N fertilization caused reduction in nodulation (Fig. 1) as was also noted by several workers (LOKRAS—TIWARI 1970, OBATON—ROLLIER 1970, SINGH 1971). The extent of reduction depended upon the level of N fertilization and stage of study. In Trial I, the reduction due to N application became conspicuous at 35 days and application of 100 kg N per ha resulted in decreased nodulation, the reduction being higher under uninoculated conditions. In other trials, the reduction in nodulation at 35 days after sowing due to N application was of ower magnitude. At 65 days after sowing, however, the magnitude of reduction became quite conspicuous and application of more than 25 kg N/ha showed a clear trend for decreased modulation and suppression was particularly striking at 200 kg N/ha or a higher level of N application.

Per hectare N uptake. In Trial I, the N uptake increased conspicuously up to 80 days after planting (Fig. 2). Thereafter, in nodulating 'Clark' there was very little increase, whereas in non-nodulating, the N accumulation declined most probably due to leaf abscission. There was small increase in the N uptake due to N fertilization in nodulating 'Clark', whereas in non-nodulating 'Clark' every successive increment in N fertilization resulted in increased N uptake (Fig. 2). For example, N fertilization resulted in an increase of 26, 56, 87 per cent and 104 per cent in the N uptake of non-nodulating 'Clark' at harvest over the control when supplied with 25, 50, 100 and 200 kg N/ha, respectively. Thus the uptake of non-nodulating 'Clark' at harvest as a function of N supply could be described by the equation: $Y = 137.6 + 14.595\ X$, where Y is the yield of N/kg/ha by non-nodulating 'Clark' for a given value of X. One X is equal to 25 kg N/ha. The correlation coefficient for this relationship was 0.925 which was highly significant.

In Trials II and III, per hectare N uptake increased till the last stage of study (Fig. 2). In Trial II, N uptake was not improved much in inoculated nodulating isolines of 'Clark' and 'Harosoy' due to N fertilization. In uninoculated nodulating and non-nodulating isolines of 'Clark', on the other hand, considerable increase was obtained due to N fertilization at most of the stages of growth. The non-nodulating 'Clark' showed an increased N uptake of 12, 85, 185, 417 and 518 per cent over the control at harvest with the application of 25, 50, 100, 200 and 300 kg kg N/ha, respectively whereas the corresponding values for non-nodulating 'Harosoy' were 16, 59, 98, 211 and 251 qer cent. At this stage, equations showing N uptake as a function of N supply for non-nodulating 'Clark' and 'Harosoy' were:

Non-nodulating 'Clark': $Y — 42.8 + 22.417\ X$

Non-nodulating 'Harosoy': $Y = 84.4 + 17.419\ X$

The respective correlation coefficients were 0.990 and 0.985, which were highly significant.

In Trial III, application of N did not affect N removal much in nodulating isolines N uptake per ha was increased considerably due to N application (Fig. 2). The uptake pattern in Trial III, therefore, differed from that in Trial II. Here uninoculated nodulating isolines behaved more like inoculated nodulating ones, rather than like non-nodulating ones. The percentage increases in N uptake at harvest over the control were 12, 19, 41, 79 and 107 per cent in non-nodulating "Clark" and 30, 104, 96, 127 and 147 per cent in non-nodulating "Harosoy" when they were given 25, 50, 100, 200 and 300 kg N/ha, respectively. The per hectare N uptake as a function of N supply was given by the following equations:

Non-nodulating "Clark": $Y = 120.3 + 10.490\ X$

Non-nodulating "Harosoy": $Y = 110.6 + 8.628\ X$

The corresponding correlation coefficients were 0.996 and 0.856, respectively, both of which were highly significant.

It is indicated that inoculated nodulating isolines did not respond to N fertilization in increasing the N uptake. In contrast, non-nodulating isolines responded considerably to N fertilization. This occurred because the latter had no source of N except fertilizer N unlike the former which had symbiosis as another N source. The uninoculated nodulating isolines behaved like inoculated nodulating isolines in Trials I and III and not in Trial II, the reason for this will be discussed later.

Contribution of symbiosis and fertilizer N equivalent. The contribution of symbiosis to total N uptake was maximum at 'no nitrogen' and 25 kg N/ha (Fig. 3 and Table 1). The highest contribution ranged from 60 to 83 per cent (182 to 247 kg N/ha) in "Clark" and 57 to 64 per cent (108 to 143 kg N/ha) in "Harosoy" under inoculated conditions. When N was applied at the highest rate, the contribution of symbiosis was reduced to a range of 12 to 20 per cent (40 to 62 kg N/ha) in "Clark" and 8 to 18 per cent (—21 to 44 kg N/ha) in "Harosoy". This shows the antagonism between symbiosis and N supply through fertilizer as far as the N nutrition of soybean was concerned. Adverse effects of increased fertilizer N on the symbiotic N yield have been observed by several workers (Nelson *et al.* 1962, Weber 1966, Trepachev *et al.* 1967). Trepachev *et al.* (1967) reported as high as 90 to 95 per cent contribution of symbiosis to total N uptake which was reduced to 40 to 70 per cent when mineral N was applied. As against this, the maximum contribution in the present study was about 57 to 83 per cent. The difference might well be due to the difference in the native N status of the soils in these two cases.

Table 2

Fertilizer N equivalent (kg/ha) to symbiotically fixed N at harvest at 'no nitrogen' and 25 kg N per ha

N levels (kg/ha)	Inoculated			Uninoculated		
	Trial I	Trial II	Trial III	Trial I	Trial II	Trial III
Clark						
0	313.2	275.8	436.2	276.5	—	351.7
25	278.5	298.4	390.6	239.1	—	390.2
Harosoy						
0	—	206.6	315.6	—	37.5	306.6
25	—	220.4	396.8	—	38.6	319.7

In the present investigation, the reduction in symbiotic N fixation due to increased N supply could be explained on the basis of the depression in nodulation due to N fertilization (Fig. 1). Tanner—Anderson (1963) suggested that the inhibition of nodulation causing reduced symbiosis might result from a nitrite-induced destruction of indolacetic acid or an ammonia-induced inhibition of conversion of tryptophan to indolacetic acid. The contribution of symbiosis to total N uptake was almost comparable under both uninoculated and inoculated conditions in Trials I and III (Fig. 3). In Trial II, on the other hand, the contribution under uninoculated conditions was far less than that under inoculated conditions. In the previous case (Trials I and III), soybean was planted in the fields where inoculated soybean was grown in the past, whereas in the latter case (Trial II) no soybean was grown in the past. The local rhizobial flora, in the first case, would have, therefore, included *Rhizobium japonicum:* whereas in the latter case, it could at the most contain *Rhizobia* of the cowpea group. The presumption is supported by the data on the nodulation obtained under these two conditions (Fig. 1). Good nodulation was obtained in Trials I and III without inoculation, whereas in the case of Trial II, very poor noculation was obtained only at a very late stage of growth. Therefore, for all practical purposes, uninoculated nodulating isolines in Trials I and III behaved like an inoculated crop, whereas in Trial II, they behaved like non-nodulating isolines. The fact, that naturalized *Rhizobia* from a previously inoculated crop of soybean were as affective in symbiosis as the directly incorporated *Rhizobia*, is of interest. However, this should not be used to recommend that yearly inoculation for soybean is not necessary. May be this type of persistence of *Rhizobium japonicum* was possible on such Tarai* soils which may have high organic matter content and good drainage. On sandy soils or soils subjected to adverse physical conditions, the survival of *Rhizobia* from the previous inoculation may not be possible.

Data on fertilizer N equivalent (Table 2) of symbiotically fixed N (Table 1) of nodulating isolines at 0 and 25 kg N per ha levels were computed because at higher levels of N application, the symbiotic N fixation was adversely affected. The need for relatively much higher amounts of fertilizer N for every unit of symbiotically obtained N is a reflection of the poor efficiency of use of the former by the soybean plant. It can be attributed to various losses to which fertilizer N is subjected in the soil during the crop season.

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* Soils of foot hills.

AMINO ACID COMPOSITION OF OPAQUE-2 KERNELS FROM DIFFERENT BACKGROUNDS

The amino acid content of maize proteins varied within certain limits according to genotype and the climatic conditions of cultivation (Bressani—Mertz 1958, Saureblich *et al.* 1953, Wolfe—Fowden 1957, Piva 1964, 1965). In general, there is an imbalance in the amino acid profile and maize protein is especially deficient in the essential amino acids, lysine and tryptophan. The total quantity of the proteins can vary according to genotype and two strains "high protein" and "low protein" have been obtained in the University of Illinois containing 22.84 per cent and 4.45 per cent protein, respectively (Leng 1961; Woodworth *et al.* 1952). Showalter—Carr (1922) found that the proteins of the "high protein" strains contain more zein than those of the "low protein" strains. Zein however is deficient in the essential amino acids, lysine and tryptophan. (Baudet *et al.* 1966, Mossé *et al.* 1966). The incorporation of the "opaque-2" gene into normal lines brings about a sensible increase in the lysine level (Mertz *et al.* 1964). With the opaque-2 gene the synthesis of zein, primarily present in the prolamin fraction of maize seed protein (alcohol soluble fraction), is partially blocked. This gives rise to an increase in the salt and alkaline soluble fractions (Mosse *et al.* 1966) and therefore a percentual increase in lysine (Mertz *et al.* 1964). This increase in lysine content is reflected in the higher nutritive value of o_{-2} maize as confirmed in experiments carried out on pigs, rats, poultry, children and adults (Mertz *et al.* 1965, Beeson *et al.* 1966, Pickett 1966, Rogler 1966, Bressani 1966, Clark 1966, Piva *et al.* 1967, Ekpenyong *et al.* 1971). In the present study, an attempt is made to search for possible background effects on the expression of the biochemical phenotype of the opaque-2 gene resulting in differences in the percentage lysine content.

Whole opaque kernels from 13 selfed hybrid lines $\times o_2$ S_5 were obtained, milled and the flour used for chemical analysis. The separation and dosage of the amino acids was done with an automated Beckman-Unichrom (Beckman Instruments) analyzer as described by Spackman *et al.* (1958). The weights of the samples for amino acid analysis ranged between 70 and 80 mg. Each sample was then hydrolyzed under nitrogen, with 5 ml 6N—HCL at 110 °C for 22h. The filtered hydrolysate was dried in a rotary evaporator. Cystine and methonine were determined as cysteic acid and methonine sulphone respectively as described by Moore (1963).

In the three tables that follow are shown the results obtained for the amino acid content in whole opaque kernels, the average values in whome kernels and the correlation between some amino acids present in higher quantities in opaque kernels as compared to normal kernels. There was no duplicate analysis and thus the variability observed for each amino acid cannot be analyzed statistically to show a possible background effect free of the experimental error.

The most important single amino acid in the work of maize protein quality improvement is lysine. For this amino acid, there is a minimum value of 3.29 per cent and a maximum value of 4.26 per cent. The significance of these differences is indispensable to the utilization such of data as a selective criterion in breeding work. Lysine is known to be the first limiting amino acid in the maize kernel and is essential from the nutritive standpoint. An increase in its content by means of breeding would be of immense value to nutritionists. The relationship existing between the percentage contents of some amino acids could indirectly serve as a pointer to the background effect. The gene, opaque-2 (Nelson 1967), induces in the endosperm a percentage increase in lysine, arginine, histidine, aspartic acid and glycine. This is due to a reduction in zein synthesis. If the experimental error is the only factor responsible for the observed variability, then there would not exist any correlations between these five amino acids. But, if on the other hand the different background effects influenced, more or less, the

Table 1

Amino acid content in whole opaque seeds obtained from selfing 13 pure lines Xo_2 (Values expressed in gm/100 gr of protein: per cent values)

Amino acid	W 153	W 22	W 3b	W 75	M 14	W 3c	W 324	W 64 A	W 374 R	Sel 224	W 187d	OH 43	W 153
Lysine	3.61	3.76	3.29	3.87	3.45	3.84	3.93	4.11	4.26	3.94	4.11	3.54	3.81
Histidine	2.56	3.07	2.67	2.66	2.76	3.12	2.83	3.02	3.29	2.79	2.83	2.88	2.96
Ammonia	11.13	5.25	6.23	4.81	5.00	5.65	5.37	5.23	6.55	5.60	5.27	5.03	4.83
Arginine	5.40	6.00	5.53	5.95	5.85	6.00	5.79	6.40	6.65	6.01	5.74	5.59	6.03
Aspartic acid	8.66	9.52	8.46	8.36	9.79	9.28	9.49	10.46	8.90	8.80	9.13	7.95	8.04
Threonine	3.66	3.56	3.46	3.59	3.80	3.81	3.76	3.49	3.66	3.50	3.65	3.59	3.51
Serine	4.79	5.00	4.87	4.71	5.11	4.97	4.50	4.27	4.49	4.22	4.43	4.16	4.16
Glutamic acid	17.19	18.75	19.09	18.73	18.73	17.26	18.10	17.47	17.32	19.10	17.83	15.43	18.67
Proline	7.96	8.53	8.53	8.67	7.69	8.77	7.32	7.43	5.98	8.36	7.26	10.89	8.67
Glycine	4.14	4.56	4.27	4.79	4.93	4.64	4.81	4.68	4.35	4.31	4.54	4.55	4.74
Alanine	5.86	6.11	6.25	6.43	6.26	6.08	6.12	5.85	6.00	6.01	5.96	5.60	6.11
Cystine	2.89	3.00	2.86	2.87	2.88	3.33	2.93	3.01	2.66	2.88	2.71	3.11	3.06
Valine	3.76	3.66	3.72	3.96	3.98	4.17	5.96	5.02	5.06	5.01	5.08	4.50	5.50
Methionine	1.95	1.78	1.97	2.09	2.37	2.04	1.91	2.03	1.93	1.68	1.49	2.11	2.32
Isoleucine	2.35	2.60	2.43	2.40	2.38	2.48	3.03	3.11	3.27	3.11	3.26	2.88	3.13
Leucine	7.83	8.51	9.09	8.75	7.12	8.14	7.67	7.93	8.39	8.20	11.05	10.47	8.30
Tyrosine	2.71	2.63	3.11	2.98	3.01	2.59	2.56	2.57	2.96	2.25	2.90	2.90	2.25
Phenylalanine	3.48	3.77	4.15	4.09	4.20	3.73	3.83	3.85	4.19	4.10	3.96	4.73	3.80
Recovery*	64.12	84.15	88.42	80.31	89.26	86.78	96.55	95.97	101.49	88.17	89.08	109.06	90.55

* Percentage amino acids recovered from total protein hydrolized. Results on this and successive table have been normalized to a recovery of 100%

Table 2

Average amino acid content in whole kernels of o_2 maize (average of 13 F_2 : g/100 g of protein)

Aminoacids	Average	Standard Error
Lysine	3.80	0.08
Histidine	2.99	0.06
Ammonia	5.84	0.48
Arginine	5.91	0.09
Aspartic acid	8.98	0.20
Threonine	3.61	0.03
Serine	4.59	0.09
Glutamis acid	17.97	0.29
Proline	8.14	0.31
Glycine	4.56	0.21
Alanine	6.04	0.06
Cystine	2.93	0.05
Valine	4.56	0.21
Methionine	1.97	0.06
Isoleucine	2.80	0.10
Leucine	8.61	0.29
Tyrosine	2.72	0.08
Phenylalanine	3.91	0.34

Table 3

Correlations between 5 amino acids present in higher proportions in normal and opaque seeds

	Histidine	Arginine	Aspartic Acid	Glycine	Aspartic ac. Glycine Histidine Arginine	Aspartic ac Glycine Histidine Arginine	Lysine Histidine Aspar. Ac. Glycine	Lysine Histidine Arginine Glycine	Lysine Histidine Arginine Aspar. ac.
Lysine	0.64*	0.74*	0.45	0.10	0.55*				
Histidine	—	0.85*	0.00	0.22		0.59*			
Arginine		—	0.39	0.21			0.67*		
Aspartic Acid			—	0.33				0.42	
Glysine									0.31

* Significant at P = 0.05

extent of the gene action, it would then be possible to obtain correlated values for the same amino acids. (Tables 1, 2).

In Table 3 are shown the values for the correlations between some amino acids. These values were those found in a larger proportion when comparing opaque and normal kernels. The results of the comparison show that the values for the correlation coefficients are always positive. In some cases, these correlation coefficients are significant when the said amino acids are arranged in possible pairs. Correlation coefficients have also been calculated between the percentage content in lysine, arginine, histidine, aspartic acid, glycine and their sum total, apart from the amino acid considered. These coefficients too are positive and in some cases significant. From the above, it could be deduced that the experimental error has only contributed a very insignificant part towards the determination of the variability of the percentage contents of each amino acid and that the differences observed could be due to the genotypic effect of the different lines.

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PRELIMINARY STUDIES ON THE VARIABILITY AMONG SCLEROTIUM CEPIVORUM BERK. ISOLATES IN THEIR TOXIN(S) PRODUCTION AND PATHOGENICITY

White rot of onion causes severe losses in upper Egypt (EL-HELALY—EL-AROSI 1964 NATTRASS 1931 and RAGAB *et al.* 1965). Variability within *Sclerotium cepivorum*, the incitant of this disease and the existence of isolates has been demonstrated by (WALKER 1924, TIMS 1948, NATTRASS 1931, and SHATLA—RUSHDI 1969). Numerous reports in the literature demonstrate the relation between toxid substances produced by plant pathogens and their pathogenicity. BRANDS (1919) showed that *Fusarium cubense*, produced a toxic substance capable of inducing the wilt of wheat plants, bean seedlings and banana leaves. BOOSALIS (1947) reported that staling products of *Rhizoctonia solani* reduced the germination of soybean seeds, and inhibited secondary root development. POUND—STAHMANN (1951) indicated that *Alternaria solani* produced metabolic substances which induce chlorosis and necrosis in tomato plants. LUKE—WHEELER (1955) showed that a toxic agent produced by *Helminthosporium victoriae* reduced the root growth of oat seedlings. *Sclerotium cepivorum* toxin(s) and their role in pathogenicity have not been investigated. This paper reports on the variability among isolates of *S. cepivorum* in toxin production, and the relation between toxin activity and pathogenicity.

Seven isolates of *S. cepivorum* were isolated from diseased onion plants from the following locations in upper Egypt: 1, Assiut; 2, Samalot; 3, Shendaweel; 4, Matay; 5, El-Menia; 6, Benisweif; and 7, El-Dawia. The isolates were maintained in pure cultures on a potato-sucrose-agar (PSA) medium. Pathogenicity tests were made by transplanting 60-day-old seedlings of "Giza—6" onion variety into clay pots containing soil non-infested (control) or infested with the test organism. Five seedlings were transplanted in each of five pots per isolate. The soil was infested by placing about 1 g inoculum in contact with the bulb and roots of each seedling at transplanting. The inoculum of each isolate was prepared in a bran onion-sand medium (BOS) (50 g wheat bran, 200 g sliced onion, 950 g washed sand plus 1000 ml distilled water), incubated at 20 °C for 21 days before use. After 60 days from transplanting, the plants were uprooted and graded (Fig. 1): 0 = healthy plants; 25 = slightly susceptible (yellowing of leaves, reduced root system); 50 = moderately susceptible (yellowing and die-back of leaves, formation of small bulbs, root system badly decayed); 75 = highly susceptible (complete yellowing of the plant, die-back of leaves, formation of small bulbs, semi-watery soft rot of scales and roots); 100 = very highly susceptible (complete death of the plant, extensive decayed bulbs and roots). The roots and bulbs of all but healthy plants were covered with mycelium and sclerotia. The disease index for each isolate was determined.

The effect of type of media, age of culture, and temperature on the toxin production, of Assiut isolate (the most virulent isolate) were studied by growing the isolate on 100 ml portions of Czapek's medium with 0.2 per cent yeast extract, and potato sucrose broth at 20 °C for 14 and 30 days. Toxin heat stability was tested by sterilizing the culture filtrates either by autoclaving at 15 lbsin. for 20 min, or by Seitz filter. Controls from uninoculated media and distilled water were used. Toxin production was assayed by determining their effect on onion seed germination and seedling dry weight. For this test five flasks, each containing a thin layer of cotton wetted with 20 ml of sterilized filtrate, were seeded with 50 surface sterilized seeds, then incubated at 20 °C for 21 days. After this period, the percentage of seed germination, and the dry weight of 10 onion seedlings (selected at random) per flask were recorded. The effect of temperature on toxin production was studied by growing Assiut isolate on Czapek's solution containing 0.2 per cent yeast extract at 15, 20 and 25 °C for 14 days. Variability among isolates in their toxin production was determined by growing the isolates on the previous medium at 20 °C for the same period. In all tests the culture filtrate was obtained and assayed as described before. The data were analyzed statistically using the analysis of variance, and the means were compared according to the Duncan multiple range test.

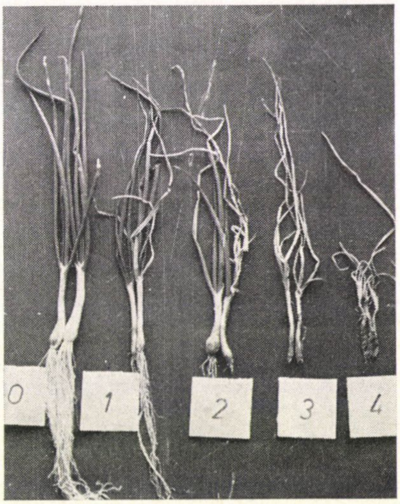

Fig. 1. Disease index. 0 = healthy plants; 1 = slightly susceptible; 2 = moderately susceptible; 3 = highly susceptible; 4 = very highly susceptible

Sclerotium cepivorum isolates varied in their pathogenicity from highly to slightly pathogenic on Giza 6 onion variety. The mean disease indices for isolates were 1 = 95.0, 2 = 73.0, 3 = 70.0, 4 = 83.0, 5 = 32.0, 6 = 70.0, 7 = 88.0. Isolates 1, 4, 7 were highly pathogenic, Isolates 2, 3, 6 were moderately pathogenic, while Isolate 5 was slightly pathogenic. Differences among isolates in their pathogenicity agreed with the finding of NATTRASS (1931), TIMS (1948) and WALKER (1924). The effect of media, autoclaving, and age of culture on toxin produc-

Table 1

Effect of media, autoclaving and age of culture on toxin production by Isolate 1

Treatments	Age of culture	Percentage of onion seed germination	Dry weight of ten seedlings (21 days old) in mg
Free sterilized, distilled water (Control 1)	—	78.50 e*	56.30 g
	—	77.75 e	57.60 g
Autoclaved, inoculated Czapek's filtrate	14-days	37.50 ab	17.80 f
	30-days	36.00 a	15.70 f
Inoculated Czapek's filtrate sterilized with Seitz filter	14-days	41.00 ab	18.60 f
	30-days	39.00 ab	13.60 f
Czapek's medium without inoculation (Control 2)	14-days	71.50 cde	47.90 g
	30-days	72.25 de	49.40 g
PSA medium without inoculation (Control 3)	14-days	72.00 de	47.20 g
	30-days	72.00 de	48.10 g
Autoclaved inoculated PSA filtrate	14-days	50.50 bc	22.70 f
	30-days	49.25 b	20.30 f
Inoculated PSA filtrate sterilized with Seitz filter	14-days	54.50 bcd	25.70 f
	30-days	52.25 bcd	19.70 f

* Means with the same letters are not significantly different

Table 2

Effect of temperature on toxin production by S. cepivorum Isolate I

Examined material	Temperatures					
	15 °C		20 °C		25 °C	
	Autoclaved filtrate	Control	Autoclaved filtrate	Control	Autoclaved filtrate	Control
Percentage of onion seed germination	36.00 a*	72.29 b	36.20 a	70.00 b	48.80	70.00 b
Dry weight of ten seedlings (21-days old) in mg	15.70 c	50.90 d	14.50 c	50.90 d	22.80	50.90 d

* Means with the same letters are not significantly different

tion are presented in Table 1. The data indicate that the composition of the tested media and the time of incubation had no effect on toxin production which agrees with WELLMANN's (1943) results on *Fusarium* sp. but not with DIMOND's (1947) observation on *Graphium ulmi.* Toxin(s) produced by S. cepivorum proved to be heat stable.

The optimum temperature for toxin (s) production was 15—20 °C (Table 2).

The variability among isolates in toxin (s) production is presented in Table 3. Isolate filtrates only varied in their toxin (s) production when assayed on the reduction of onion seed germination. The culture filtrate from Isolate 5 was weakly toxic, from Isolates 2, 3 were highly toxic, while those from Isolates 1, 4, 6, 7 were moderately toxic. The compared data of toxin production and pathogenicity indicate that no obvious correlation was found between them.

*

Table 3

Variability among Sclerctium cepivorum isolates in their toxin production

Examined material	Isolates							Control
	I	II	III	IV	V	VI	VII	
Percentage of onion seed germination	38.75 bc*	34.25 a	37.50 ab	40.00 bc	48.25	42.00 C	38.25 bc	73.25
Dry weight of ten seedlings (21-days old) in mg	15.70 d	18.40 d	16.70 d	18.00 d	33.70 d	20.90 d	14.10 d	50.22

* Means with the same letters are not significantly different

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EFFECT OF SUBMERGENCE ON THE PHYSICO-CHEMICAL AND CHEMICAL CHANGES IN DIFFERENT RICE SOILS I. KINETICS OF pH, Eh, C AND N

Continuous submergence of the soil as obtained under rice growing conditions, sets in motion, a series of physical, physico-chemical, chemical and microbiological changes which are quite different from those of aerated soils growing other cereals like wheat, maize, etc. Information has been reported from different rice growing countries of the world, on the nature and magnitude of these changes under respective conditions. Very little attempt has, however, been made to quantify these changes for the purpose of prediction. Anonymous (1964) has reported equations relating to the transformation of nutrients with initial edaphic factors. Attempts were made in a series of experiments conducted by the authors to quantify some physico-chemical and chemical changes as a function of time in terms of prediction equations and evaluate various inter-relationships of these transformations.

Laboratory incubation experiments were conducted with 20 surface soil samples of representative rice growing tracts of the country, to study the changes in pH, Eh, C, N, P and cations like K, Ca, Mg, Fe and Mn as a function of the time of submergence. The characteristics of the soils used in these investigations have been reported in detail by Mohanty *et al.* (1974). Some of the important characters of these soils are given in Table 1.

Fifty gram lots of 2 mm air-dried samples, contained in 250 ml conical flasks, were submerged with 50 ml distilled water and incubated in dark at 30° ± 2 °C. Duplicate sample flasks from each soil were drawn at the start of the experiment and at intervals of 10, 20, 30, 50 and 70 days of submergence, extracted with N NaCl and the extracts were analyzed for oxidizable organic matter (Rodrigo 1961), NH_4—N by steam distillation in the presence of NaOH.

Table 1

Characters of soils used in the experiment

Soil No.	Location	Soil Type	pH	Clay%	C%	N%	CEC m. e/100 g soil
1.	Bhubaneswar (Orissa)	Laterite	4.7	20.7	0.5	0.06	8.5
2.	Sukinda (Orissa)	Laterite	5.6	37.9	0.4	0.08	12.2
3.	Pattambi (Kerala)	Laterite	5.2	33.7	2.0	0.22	18.2
4.	Nagenahalli (Mysore)	Laterite	6.4	28.2	1.4	0.13	28.2
5.	Berhampur (Orissa)	Red loam	5.0	36.6	0.6	0.07	11.5
6.	Chakuli (Orissa)	Red loam	6.9	29.2	1.0	0.05	22.3
7.	Parmanpur (Orissa)	Red loam	5.9	21.6	0.7	0.02	11.1
8.	Gamharipalli (Orissa)	Red loam	6.2	16.4	1.5	0.05	19.4
9.	Chiplina (Orissa)	Red loam	6.0	25.6	1.4	0.06	18.6
10.	Bargarh (Orissa)	Red loam	5.9	24.8	1.0	0.05	24.4
11.	Barpali (Orissa)	Red loam	5.7	24.9	1.1	0.04	21.1
12.	Cuttack (Orissa)	Alluvial	5.3	35.6	0.5	0.06	21.1
13.	Sakshigopal (Orissa)	Alluvial	5.3	49.3	0.8	0.10	28.2
14.	Kujang (Orissa)	Alluvial	6.2	30.9	0.6	0.06	32.8
15.	Kendrapara (Orissa)	Alluvial	5.1	51.9	0.6	0.06	40.3
16.	Bolangir (Orissa)	Black	7.9	57.1	1.0	0.12	49.2
17.	Arkabahali (rissa)	Black	6.6	44.3	1.8	0.05	63.9
18.	Palur (Tamil Nadu)	Black	7.8	28.9	0.5	0.06	36.9
19.	Nellore (Andhra Pradesh)	Black	8.5	29.1	0.6	0.07	37.1
20.	Keshpur (Orissa)	Coastal Saline	6.7	44.8	0.3	0.04	42.2

For determining the change in pH and Eh, 50 g lots of soil were taken in 100 ml beakers, submerged with 50 ml distilled water and incubated. At specified intervals, the pH was determined with a glass electrode and a redox potential with a bright Pt electrode in a Beckman Model H2 pH meter. From the observed pH and Eh values expressed in volts, the Eh_6 values were calculated using the equation (Aomine 1962).

$$Eh_6 = Eh - 0.06\,(6 - pH)$$

Table 2

Equations for change in pH and Eh with period of submergence in different soils

Soil No.	Equation best fitted	'a'	'b'	'c'	R^2 %
6	$y = a + bt + ct^2$	6.99	—0.17	0.02	63.95
17	-do-	6.89	—0.18	0.02	90.14
20	-do-	6.67	—0.05	—0.05	90.06
16	$y = a + b \ln (t + l) + c[\ln (t + l)]^2$	7.88	—1.22	0.23	92.52
18	-do-	7.81	—1.41	0.53	95.01
19	-do-	8.45	—1.70	0.66	74.49
1	$\ln y = a + b \ln (t + l) + c[\ln (t + l)]^2$	1.76	0.52	—0.26	90.89
2	-do-	1.90	0.10	—0.07	75.85
3	-do-	1.83	0.34	—0.33	93.53
4	-do-	2.0	0.13	—0.05	95.12
5	-do-	1.81	0.30	—0.15	77.65
7	-do-	1.95	0.06	—0.08	88.96
8	-do-	1.99	0.14	—0.08	85.07
9	-do-	1.96	0.16	0.06	78.39
10	-do-	1.94	0.10	—0.07	79.04
11	-do-	1.92	0.19	—0.09	72.46
12	-do-	1.89	0.06	—0.04	72.65
13	-do-	1.85	0.27	—0.15	93.71
14	-do-	1.99	0.15	—0.07	79.25
15	-do-	1.81	0.03	—0.05	90.51

Soil No.	Equation best fitted	'a'	'b'	'c'	R^2 %
14	$y = a + bt + ct^2$	221.02	—169.41	23.48	53.07
1	$y = a + b \ln (t + 1) + c [\ln (t + l)]^2$	208.39	—459.38	263.52	66.05
2	-do-	190.83	—175.27	112.66	73.96
3	-do-	233.96	—686.80	274.67	90.40
4	-do-	186.56	—785.72	327.25	96.00
5	-do-	233.04	—284.41	114.46	23.84
6	-do-	177.53	—481.83	249.44	75.47
7	-do-	237.60	—400.72	196.97	91.91
8	-do-	155.73	—378.12	205.57	63.12
9	-do-	202.32	—438.49	215.91	81.18
10	-do-	200.62	—251.43	146.15	83.27
11	-do-	206.33	—251.43	146.15	67.31
12	-do-	244.76	—160.41	83.18	50.71
13	-do-	198.23	—396.41	222.76	63.74
15	-do-	198.58	— 66.11	75.30	87.40
16	-do-	145.54	—359.54	188.95	87.32
17	-do-	196.27	—334.02	172.58	70.04
18	-do-	175.35	—640.89	300.72	97.20
19	-do-	153.44	—751.72	340.02	96.75
20	-do-	209.40	—208.49	109.57	65.13

The data were processed in an IBM 1620 computer to fit the kinetics in linear, quadratic, logarithmic and exponential functions.

Of the four functions fitted for each parameter, the one with the highest R^2 value has been selected and summary tables on these prediction equations have been presented, giving their nature, 'a', 'b', 'c' and R^2 values where 'Y' is the value of the parameter studied at the

Table 3

Equations for change in oxidizable organic matter and available NH_4—N in the NaCl extract with period of submergence in different soils

Oxidizable organic matter

Soil No.	Equations best fitted	'a'	'b'	'c'	R² %
2	$y = a + bt + ct^2$	0.27	—0.05	0.003	94.31
3	-do-	7.62	1.43	—0.34	98.57
4	-do-	6.71	—0.39	—0.05	91.80
7	-do-	0.22	0.02	—0.004	23.69
10	-do-	0.80	—0.15	0.04	70.89
14	-do-	1.31	—0.45	0.05	98.75
16	-do-	0.21	0.12	—0.01	32.50
17	-do-	0.13	0.13	—0.02	45.75
19	-do-	3.02	—1.05	0.10	98.23
1	$\ln y = a + b \ln (t + l) + c[\ln(t + l)]^2$	0.32	1.45	—0.71	79.44
5	-do-	0.26	0.47	—0.18	60.88
6	-do-	0.20	0.61	—0.20	84.77
8	-do-	0.34	0.22	—0.12	34.22
9	-do-	0.36	1.32	—0.48	85.53
11	-do-	0.41	1.04	—0.36	80.39
12	-do-	0.10	0.21	—0.11	65.77
13	-do-	0.15	0.69	—0.31	89.32
15	-do-	0.19	0.24	—0.15	32.76
18	-do-	1.52	—1.36	0.44	96.14
20	-do-	1.65	—2.15	0.72	94.63

Available NH_4—N

	Equation best fitted	'a'	'b'	'c'	R² %
3	$y = a + bt + ct^2$	93.13	18.92	—4.93	98.72
4	-do-	82.59	— 4.87	—0.65	91.92
6	-do-	37.25	—10.87	0.89	82.76
10	-do-	75.40	—22.37	1.84	95.55
14	-do-	16.47	— 5.66	0.58	99.18
19	-do-	36.90	—12.87	1.18	98.06
2	$y = a + b \ln (t + l) + c[\ln (t + l)]^2$	3.81	— 0.71	—0.02	63.29
15	-do-	13.48	17.68	—10.59	90.73
17	-do-	23.25	—17.46	4.30	98.22
1	$\ln y = a + b \ln (t + l) + c[\ln (t + l)]^2$	1.71	1.28	—0.78	89.08
5	-do-	1.55	0.70	—0.39	52.90
7	-do-	3.16	1.52	—1.05	81.78
8	-do-	4.30	0.20	—0.59	91.19
9	-do-	3.66	0.62	—0.62	85.78
11	-do-	4.04	0.03	—0.47	87.48
12	-do-	1.78	0.71	—0.40	87.22
13	-do-	3.31	0.99	—0.85	98.32
16	-do-	3.47	—1.07	0.08	99.44
18	-do-	3.80	—2.02	0.62	96.78
20	-do-	3.99	—3.78	1.20	96.18

time of submergence of 't' days, 'a' is a constant and 'b' and 'c' are the regression coefficients. For details on soil numbers, please refer to Table 1.

Changes in soil pH. Fourteen of the 20 soils showed the exponential pattern of pH change (Table 2). There was a general tendency of pH to increase during the first 10 days of submergence after which there was a decrease to values even sometimes lower than the initial pH. The change in pH was more pronounced in the laterite and red loam soils. In general, these soils were slightly to moderately acidic, low to medium in exchangeable Ca and Mg and high in organic C.

The pH of soils 16, 18 and 19, which showed a logarithmic function, decreased rapidly during the first 10 days and then slowly during the next 60 days. Soils had initial pH of 7.8 to 8.5 and were medium in exchangeable Ca and Mg. Three neutral soils (pH 6.7 to 6.9) followed the quadratic type of change where the pH remained more or less stationary during the first 20 days and then slightly decreased.

Change of pH was found to be positively correlated with the initial soil pH, exchangeable Ca and change in exchangeable Mg and negatively correlated with the dynamics of Eh and water soluble P.

Change in redox potential. All soils except No. 14 showed logarithmic change in the redox potential (expressed as mv) as a function of time of submergence (Table 2). In soils with high organic C and total N, there was a rapid decrease in the Eh to negative values during the first 20 days of submergence after which there was a slight increase whereas those with low to moderate organic C also showed rapid decrease during the first 20 days, but in these soils, negative Eh values were not obtained. After 20 days, there was again a rise in Eh. The change in Eh was found to be negatively correlated with the initial soil pH, the change in pH due to submergence and the amount of readily oxidizable organic matter.

Change in oxidizable organic matter. The change in oxidizable organic matter in the NaCl extract (expressed as ml of 0.05 N $KMnO_4$) was quadratic or exponential (Table 3). In most situations, there was an increase in this parameter during the first 20 days of submergence followed by a decrease. The transformations of oxidizable organic matter were found to be positively correlated with total N, organic C and the change in NH_4—N and negatively correlated with the change in redox potential as a function of time of submergence.

Change in available ammonium nitrogen. Six of the 20 soils studied showed quadratic change in the available NH_4—N (Table 3) expressed as ppm. In these soils, there was an increase in NH_4—N during the first 20 days after which there was a sharp decline. These soils were rich in C, N and had low clay content and cation exchange capacity. Three soils with low C, N and high clay and cation exchange capacity showed logarithmic change in the available NH_4—N where the values of NH_4—N were high during the first 10 days after which there was a gradual decline. The rest of the soils exhibited an exponential change in available N where the increase was sharp during the first 30 days after which there was no further change. These soils had medium C, N, clay and cation exchange capacity. The dynamics of available was found to be positively correlated with total N, C and the change in oxidizable organic matter with time.

The results presented show the changes in pH, Eh, C and N in different soil types as influenced by the periodic of submergence. The increase in pH in acid soils was due to the accumulation of ammonia and the change in the ferrous–ferric equilibrium (PATRICK 1964, PONNAMPERUMA *et al.* 1966) whereas the decrease in pH in soils with initial pH of 7.8 to 8.5 migh be attributed to the pCO_2 through the Na_2CO_3—H_2O—CO_2 and the $CaCO_3$—H_2O—CO_2 systems (PONNAMPERUMA *et al.* 1966) and to the removal of Na_2CO_3 by Ca^{++} and Mg^{++} (ROMANOFF 1945). The decrease in pH after reaching a peak in the acid soils has been explained by RODRIGO (1962) to be due to diminishing concentrations of NH^+, Fe^{++} and Mn^{++} with lapse of time.

The changes in NH_4—N, oxidizable organic matter and redox potential appeared to be very much inter-related. The increase in NH_4—N with the period of submergence was due to mineralization and so the magnitude available was dependent on the total C and N. Higher content of organic matter resulted in a sharp decrease in the redox potential, to negative values in many situations. These corroborate the earlier reports (Anonymous 1963, Rodrigo 1962, 1967). At later periods of submergence, there was decrease in the amount of NH_4—N which might be due to microbial assimilation and denitrification.

These changes have been quantified in terms of various equations for the individual soils. Based on the patterns obtained, the following generalizations may be made for predicting the changes.

pH:

$Y = a + bt + ct^2$	Neutral soils
$Y = a + b\ \ln(t + 1) + c[\ln(t + 1)]^2$	alkaline soils
$\ln Y = a + b\ \ln(t + 1) + c[\ln(t + 1)]^2$	acid soils
Eh (mv):	
$Y = a + b\ \ln(t + 1) + c[\ln(t + 1)]^2$	most soils
NH_4—N (ppm):	
$Y = a + bt + ct^2$	soils with high C, N; medium clay, CEC
$Y = a + b\ \ln(t + 1) + c[\ln(t + 1)]^2$	soils with low C, N; high clay, CEC
$\ln Y = a + b\ \ln(t + 1) + c[\ln(t + 1)]^2$	soils with medium C, N clay, CEC

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PHYSIOLOGICAL STUDIES ON SALT TOLERANCE IN PISUM SATIVUM (L.)

III. Growth and maturation

Salt stress in the soil is experienced by most of the crop plants as saline soils are widely distributed in the arable lands of India. It is, therefore, apparent that enhancement in the capacity of plants to resist the presence of large amounts of salts in their rhizosphere will certainly be of immense importance. In this respect considerable interest has been aroused in the growth modifying responses of gibberellins and growth retarding chemicals. It has been observed that besides altering growth, without injurious effects, growth regulators also help plants in tolerating conditions such as salinity stress (Marth—Frank 1961, Miyamoto 1963, El Damaty *et al.* 1964, Sarin—Uprety 1965 and Nieman—Bernstein 1959). In the present investigation, therefore, an attempt was made to analyze the interactive effect of soil salinity and growth regulators (gibberellic acid and phosphon-D) on the growth and development of pea plants.

Two varieties of *Pisum sativum* (L.), i.e. '*Rimpus*' and '*Vares*' were grown in earthenware pots lined with impervious alkathene sheets (to prevent leaching of salts). Two series of these pots were maintained one with unsalinized soil (electrical conductivity (E. C.)of the soil saturation extract was 3.5 mmhos/cm at 25 °C) and another with artificially salinized soil (E. C. of soil saturation extract was 8.5 mmhos/cm at 25 °C). The salinization of the soil was carried out by adding an equal proportion of sodium chloride and calcium chloride as reported in an earlier communication. In the salinized soil series four groups of 25 pots were maintained for the following treatments: (i) Plants sprayed with 10 ml of 1.3×10^{-4} M phosphon-D solution per plant, (ii) Plants sprayed with 10 ml of 1.4×10^{-4} M gibberellic acid solution per plant, (iii) Plants sprayed with 5 ml of each of these two chemicals (same concentration) per plant, (IV) Unsprayed plants. Plants grown in unsalinized soil were not sprayed. Various concentrations of these growth regulators were tested and it was found that with the aforesaid doses, these chemicals showed their maximum effect on growth without any injurious influence (Uprety 1971). The spraying commenced 15 days after sowing to coincide with the salinization of the soil. In case of combined spray the plants were treated first with phosphon-D and after 4 hours with the gibberellic acid solution. In all four groups fortnightly sprays were given in each treatment combination.

Observations on the height of plants, number of leaves and dry weight of plant parts were recorded periodically at an interval of 15 days from 20 to 80 days after sowing and the absolute rate of elongation (cm/day), coefficient of velocity of leaf production (leaf/plant/day) and relative growth rate (g dry matter/plant/day) of the individual plant parts were calculated similarly as reported by Sarin—Uprety (1965). Final plant height, leaf and branch number were also tabulated for reference. In order to understand the distribution of dry matter in the various plant parts, the dry weight of roots, stem and leaves as a percentage of the dry weight of the whole plant (root + stem + leaves) was calculated.

Observations on the following flowering characters were also recorded.

Days to flowering were calculated from the sowing time to the first visual appearance of the flower. The number of flower buds, flowers and fruits were periodically counted and their total number per plant were calculated. The percentage fertility of flowers was determined on the basis of the number of flowers which formed fruits. Days to maturation of fruits were calculated from flowering upto the harvest of the fruits. Fruit size was measured by the length. Other data recorded were total number of seeds per plant, size of seeds (diameter of seeds — measured with screwguage), hundred seed weight and seed yield per plant.

All the growth, flowering and yield data were analyzed statistically following the analysis of variance method. The experiment was repeated three times with similar results and therefore, only the data of one replication are presented for the sake of brevity.

Vegetative growth

(i) Soil salinity significantly decreased the height of plants and absolute rate of elongation in both the varieties. The reduction was more in var. 'Rimpus'. Treatment with gibberellic acid (GA_3) as well as a combined spray of GA_3 and phosphon-D ameliorated the adverse effect of soil salinity on the elongation growth. This response was more marked in var. 'Vares'. Application of phosphon-D did not significantly alter the elongation of the plant (Table 1 (i) and (ii)).

(ii) Soil salinization affected the leaf production adversely and this effect was more pronounced in var. 'Vares'. It was observed that the application of phosphon-D considerably increased the production of leaves in plants grown under salinized conditions. Other growth

Table 1

Effect of soil salinity and growth regulators on the vegetative growth of Pisum sativum plants

Characters	Variety	Unsalinized soil	Salinized soil				C.D. AT 5% P
		Unsprayed plant	Unsprayed plant	Phosphon-D sprayed plant	GA_3 sprayed plant	GA_3+ phosphon-D sprayed plant	
(i) Final height of plant (CM)	Rimpus	53.5	38.2	32.5	50.8	46.5	6.7
(80 Days after sowing	Vares	17.8	15.2	14.0	40.0	33.7	2.3
(ii) Absolute rate of elongation	Rimpus	0.674	0.488	0.403	0.696	0.562	0.121
(CM/plant/day)	Vares	0.202	0.178	0.167	0.497	0.420	0.099
(iii) Total number of leaves (per plant)	Rimpus	17.7	13.0	17.7	12.4	13.8	2.71
(At 80 days after sowing)	Vares	41.2	29.4	38.0	24.9	22.4	7.62
(IV) Coefficent of velocity of leaf	Rimpus	0.217	0.165	0.232	0.154	0.175	0.018
leaf production (leaf/plant/day)	Vares	0.538	0.370	0.484	0.312	0.278	0.110
(V) Total number of branches	Rimpus	2.6	1.8	3.4	1.8	2.2	N.S.
(at 80 days after sowing)	Vares	5.4	4.4	6.0	2.8	4.2	N.S.
(VI) Relative growth rate	Rimpus	0.0376	0.0251	0.0358	0.0197	0.0296	0.0046
(g. dry weight/plant/ day)							
(A) *Leaf*	Vares	0.0394	0.0309	0.0395	0.0315	0.0294	0.0035
	Rimpus	0.0200	0.0112	0.0140	0.0169	0.0120	0.0027
(B) *Stem*	Vares	0.0176	0.0145	0.0149	0.0198	0.0132	0.0030
	Rimpus	0.0048	0.0103	0.0106	0.0105	0.0101	0.0031
(C) *Root*	Vares	0.0053	0.0110	0.0105	0.0103	0.0099	0.0027
	Rimpus	0.0624	0.0466	0.0604	0.0471	0.0517	0.0038
(D) *Total plant*	Vares	0.0623	0.0564	0.0649	0.0628	0.0505	0.0047

[+] N. S. Non significant

(VII) Percentage distribution of dry matter in plant vars. rimpus vares
(Values in parentheses are those of var. vares)

Soil type	Treatments	Plant parts	Days after sowing				
			20	35	50	65	80
Unsalinized soil	Unsprayed plant	leaf	54 (60)	46 (45)	55 (56)	61 (64)	62 (63)
		stem	17 (16)	35 (38)	33 (29)	31 (28)	31 (27)
		root	29 (24)	19 (17)	12 (15)	8 (8)	7 (10)
Salinized soil	Unsprayed plant	leaf	40 (45)	40 (41)	41 (42)	55 (56)	56 (59)
		stem	10 (8)	17 (21)	25 (27)	21 (24)	24 (25)
		root	50 (47)	43 (38)	34 (31)	24 (20)	20 (16)
	Phosphon-D sprayed plant	leaf	54 (48)	49 (41)	47 (50)	63 (60)	64 (61)
		stem	14 (10)	23 (27)	22 (26)	21 (25)	22 (28)
		root	32 (42)	28 (32)	31 (24)	16 (15)	14 (11)
	GA_3 sprayed plant	leaf	25 (39)	27 (30)	27 (31)	42 (44)	47 (45)
		stem	25 (20)	36 (38)	36 (38)	37 (37)	35 (39)
		root	50 (41)	37 (32)	37 (31)	21 (19)	18 (16)
	GA_3 + phosphon-D sprayed plant	leaf	35 (42)	41 (37)	50 (41)	59 (50)	62 (57)
		stem	15 (8)	23 (25)	21 (26)	22 (28)	23 (27)
		root	50 (50)	36 (38)	29 (33)	19 (22)	15 (16)

regulator treatments, however, did not appreciably influence the leaf production (Table 1 (iii) and (IV)).

(iii) There was no significant influence of either soil salinity or growth regulators on the number of branches (Table 1 (V)).

(IV) Soil salinization significantly lowered the relative growth rate (RGR) of the whole plant, leaves and stem but increased that of the roots in both the varieties. Application of phosphon-D brought about a marked enhancement in the RGR of the whole plant and leaves whereas the RGR of the stem was increased by the GA_3 treatment. None of the growth regulator treatments, however, influenced the RGR of the roots (Table 1 (VI)).

(V) The distribution of dry weight in the different plant organs revealed that there was a steady decline in the proportion of dry weight in the roots with the age of the plants. The dry weight of the stem remained more or less constant and that of the leaves constantly increased with age.

Soil salinity brought about a considerable accumulation of dry matter in the roots and the proportion of stem and leaves decreased. Application of phosphon-D to plants reduced the salinity induced accumulation of dry matter in the roots. Other growth regulator treatments did not alter the pattern of distribution (Table 1 (VII)).

Table 2

Effect of soil salinity and growth regulators on the maturation of pisum sativum plants

Characters	Variety	Unsalinized soil	Salinized soil				C.D. AT 5% P
		Unsprayed plants	Unsprayed plants	Phosphon-D sprayed plants	GA_3 sprayed plants	GA_3+ phosphon-D sprayed plants	
(I) days to flower	rimpus	52.00	64.42	55.46	67.10	53.20	4.01
	vares	65.10	69.40	61.20	64.60	61.60	4.27
(ii) number of flower buds	rimpus	18.20	12.60	23.00	10.80	11.80	2.71
	vares	34.00	28.20	38.40	26.60	28.00	4.20
(iii) number of flowers	rimpus	13.48	8.54	16.80	6.98	7.00	1.58
	vares	30.20	24.40	31.20	21.80	23.80	2.28
(IV) number of fruits	rimpus	7.10	4.10	9.36	3.20	5.46	1.49
	vares	20.10	9.60	20.020	10.20	12.20	1.66
(V) fertility of flowers %	rimpus	52.60	48.00	55.70	45.80	60.00	3.70
	vares	66.50	39.30	64.70	46.70	51.20	9.20
(VI) days to maturation of fruits	rimpus	31.50	37.00	28.50	36.50	35.00	3.20
	vares	28.30	36.00	27.50	33.00	34.00	3.40
(VII) length of fruit (Cms)	rimpus	7.08	5.92	5.80	4.94	6.06	0.38
	vares	6.04	4.98	5.44	4.06	3.90	0.42
(VIII) number of seeds per plant	rimpus	24.60	18.00	29.00	13.80	21.00	4.05
	vares	38.00	31.00	44.00	25.60	32.60	4.31
(IX) size of seed (mm)	rimpus	6.898	4.816	5.140	5.742	4.870	0.36
	vares	6.614	4.160	4.190	4.858	4.166	0.62
(X) hunderd seed weight (G)	rimpus	28.43	21.77	22.40	24.41	22.72	1.83
	vares	21.12	18.24	14.74	20.08	17.66	1.33
(XI) seed yield (g)/plant	rimpus	7.16	3.26	6.80	2.40	3.88	0.71
	vares	8.26	6.20	8.40	6.16	6.56	0.69

Flowering and yield

Soil salinity significantly delayed the flowering in both the varieties. It was observed that the salt induced delay in flowering was counteracted in phosphon-D and GA_3 and phosphon-D treated plants. The effect of GA_3 was not marked (Table 2 (i)).

Soil salinity significantly hampered the production of flower buds, flowers, fruits and the fertility of flowers in both the pea varieties. The phosphon-D treatment brought about a marked improvement in these flowering attributes. Application of gibberellic acid did not appreciably influence these characters. The combined spraying of GA_3 and phosphon-D improved the fertility of the flowers in both the varieties and the production of fruits in var. 'Vares' (Table 2, II, III, IV and V).

Maturation of fruits was considerably delayed by soil salinity. The phosphon-D treatment to plants brought about a marked earliness, however the effect of GA_3 or a combination of GA_3 and phosphon-D was not significant (Table 2, VI).

Length of fruits was adversely affected by soil salinity in both var. 'Rumpus' and 'Vares'. Growth regulator treatments, however, did not influence this character (Table 2, VII).

Not only flowers and fruits but the production of seeds was also significantly reduced by soil salinity. The phosphon-D treatment increased while the GA_3 decreased the number of seeds per plant (Table 2, VIII).

Soil salinity adversely affected the size of seeds as well as the hundred seed weight. Application of GA_3 brought about a significant improvement in the size and weight of seeds. Other treatments did not appreciably alter these characters (Table 2, IX and X).

Soil salinity significantly lowered the seed yield of plants. Phosphon-D spray to plants markedly ameliorated this adverse effect in both the varieties. GA_3 and a combined treatment of GA_3 and phosphon-D did not bring about any improvement in this character (Table 2, XI).

The adverse effect of soil salinity on vegetative growth has been observed both as reduced height as well as lowered production of leaves in various crop plants (WADLEIGH—GAUCH 1944, BERNSTEIN—AYERS 1951, SARIN—UPRETY 1965 and GATES *et al.* 1970). The results of the present study on peas also confirmed the above observations and revealed that these responses were apparently the result of the lowered elongation rate and reduced velocity of leaf production culminating in the lower relative growth rate of the plants. Variety Rimpus was more susceptible to salt damage for elongation and var. 'Vares' for the production of leaves. It was interesting to observe that the salt induced depression in the RGR of whole plant was primarily because of the lowered rate of growth in the leaves and stem and not in the roots. It can also be evidenced from the observations on the distribution pattern of dry matter in the plant parts. MARTH—FRANK (1961) in soybean, EL DAMATHY *et al.* (1964) and MIYAMOTO (1963) in wheats reported induction of salt tolerance by cycocel treatment. Their conclusions were based on the observation that cycocel treated plants did not wilt under saline conditions. In the present study, however, application of phosphon-D counteracted the adverse effect of soil salinity on the RGR of plants by increasing the photosynthetic surface as measured by the number of leaves. Application of gibberellic acid alone as well as in combination with phosphon-D helped in improving elongation growth and this response was more pronounced in var. 'Vares'. The GA_3 treatment did not bring about any beneficial effect on the leaf production and relative growth rate of plants. This response supports the findings of NIEMAN—BERNSTEIN (1959) that at high salinity there is no improvement in growth due to GA_3 application.

The adverse effect of soil salinity on growth was also reported to be carried over to the development of plants (HAYWARD—LONG 1943, BERNSTEIN—AYERS 1951 and SARIN—UPRETY 1965). The above authors contended that reduction on yield was due to a lowered flowering. In the present investigation with peas, the deleterious effect of soil salinity during development was manifested not only in a reduced flowering but also decreased seed size and seed weight. Further the adverse effect was also observed as delay in flowering and maturation of fruits as well as lowering in fertility of flower, number and size of fruits, and finally seed yield.

The exogenous application of phosphon-D ameliorated the harmful effect of salinity on the above mentioned developmental attributes. An increase in flowering due to the growth retardant CCC has been observed in tomato (WITWER—TOLBERT 1960) and due to B-nine in trees (CATHEY 1964). As suggested by CATHEY (1964) the growth retardants may promote flowering by modifying the activity of the cambium resulting in the restriction of elongation growth which presumably alters the metabolism and creates conditions condusive to flowering. The treatment with gibberellic acid, however, failed to improve the adverse effect of salinity on flowering and yield characters except seed weight and seed size. The combined application of GA_3 and phosphon-D did not bring about any appreciable change in the yield characters.

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POSITION OF MAIZE IN THE ROTATION

I. Effect of preceding winter crops and nitrogen fertilization on some agronomic characters of maize

Maize is an important cereal crop in Egypt. It is used for human consumption and in feeding animals. Maize is a summer crop which is grown after leguminous and non-leguminous crops. It is a nitrogen loving crop which grows and thrives better at high nitrogen levels.

The effect of preceding crops on the yield and growth of succeeding ones has attracted the attention of many investigators. CARTMILL (1953) reported that maize following groundnuts yielded 25—40 per cent more than maize following maize. He added that the percentage of barren stalks decreased with the nitrogen application. MOURSI *et al.* (1965) found that the yield of peanut plants after field beans significantly outweighed that of plants grown after barley, flax and wheat. They added that the preceding crop had no significant effect on the average number of leaves per peanut plant; but the number of fruits per plant after barley was inferior to that after field beans, berseem and flax. KÖNNECKE (1967) reported that the protein content and 1000-kernel weight of wheat, barley and oats were affected by the preceding crop. He found that protein content in cereals increased after legumes, while the 1000-kernel weight of cereals decreased when they were preceded by legumes. SHORMA—SINGH (1969) arranged the yield of maize according to the effect of the preceding crop in a descending order as follows: berseem, sweet clover, peas and fallow. Yield increases after legumes and after applying nitrogen were ascribed to increases in grain test weight, weight of grain per cob and cob length.

The effect of nitrogen on the growth and yield of maize has been studied by many investigators. WOLFE (1924) found that the yield of maize, weight of grains per ear, shelling percentage and ear length increased significantly with the addition of nitrogen. COLWELL (1947), CARTMILL (1953), HUSSEIN (1958) and SHAFSHAK—EL-DEBABY (1971) reported that the application of nitrogen considerably decreased the percentage of barren plants in maize.

Table 1

Mean percentage of lodged plants
(Combined analysis of 1969, 1970 and 1971 seasons)

Nitrogen Kgs/ha	Preceding winter crop					Mean
	Flax	Wheat	Barley	Beans	Berseem	
0	23.88	19.60	19.44	16.07	11.86	18.17 a
74	8.06	7.32	11.38	8.86	6.19	8.36 b
148	7.48	5.59	10.02	15.49	9.37	9.59 b
Mean	13.14 a	10.84 a	13.61 a	13.47 a	9.11 a	12.04

The effect of nitrogen on lodging has been studied by many investigators. KRANTS—CHANDLER (1951), ZUBER *et al.* (1954), FISHER—SMITH (1957) and HUSSEIN (1968) reported that nitrogen increased lodging in maize, while HUSSEIN (1958) found that nitrogen had very little effect on lodging.

According to HUSSEIN (1958) and HUSSEIN (1968), the percentage of broken maize plants increased due to the application of nitrogen.

Nitrogen had also considerable effect in increasing the number of ears per plant in maize (HUSSEIN 1958, RAI 1961, SHAFSHAK—EL-DEBABY 1971). VITTUM (1948) came to another conclusion, where he found that the number of ears per plant was not increased by nitrogen application due to the large size of ears in nitrogen treated plots.

Nitrogen showed also a good effect on the 100-kernel weight of maize as reported by HUSSEIN (1958), RAI (1961) and SHORMA—SINGH (1969), while KHALIFA (1970) found that the effect of nitrogen on the weight of 100-kernels was not significant.

This study was intended to investigate the effect of preceding winter crops as well as the different rates of nitrogen fertilizer in avoiding some problems in maize production such as lodging, breakage and barren plants and on some characters related to the yield such as number of ears per plant, shelling percentage and weight of 100-kernels.

Three experiments were conducted at the Higher Institute of Agriculture, at Moshtohor, Kalubia Governorate, in 1969, 1970 and 1971 to study the effect of preceding winter crops and nitrogen fertilizer on the lodging, breakage, barren plants, number of ears per plant and shelling percentage of maize. Each experiment included 15 treatments which were the combinations of five preceding crops, namely: — Flax (*Linum usitatissimum* L.) variety Giza 4, — Wheat (*Triticum aestivum* L.) variety Giza 155, — Barley (*Hordeum vulgare* L.) variety Giza 117, — Field beans (*Vicia faba* L.) variety Giza 2, and — Berseem (*Trifolium alexandrinum* L.)

variety Meskawy, and three levels of nitrogen fertilizer, namely: control, 74 and 148 kgs nitrogen per hectare.

The experiments were designed according to split plot design with five replications. The preceding crops were arranged at random in the main plots, while the nitrogen rates were also arranged at random in the sub-plots. The area of the main plot was 63 sq. meters and that of the sub-plot was 21 sq. meters.

The preceding crops were given their normal cultural treatments and fertilizers. The maize was planted in rows, 30 cms apart, each row was 70 cm wide. Planting dates were May 25, May 12 and June 18 in 1969, 1970 and 1971, respectively. Double Cross 67 cultivar was grown in 1969 and 1970, while Double Cross 17_S was used in 1971. Fertilizers were side dressed in split application. Harvesting started after 129, 133 and 119 days from planting in 1969, 1970 and 1971, respectively.

Table 2

Mean percentage of broken plants
(Combined analysis of 1969, 1970 and 1971 seasons)

Nitrogen kgs/ha	Preceding winter crop					Mean
	Flax	Wheat	Barley	Beans	Berseem	
0	16.86	12.91	20.44	22.05	18.19	18.09 a
74	15.97	16.43	17.77	20.33	18.34	17.77 a
148	16.98	12.52	16.31	22.38	18.27	17.29 a
Mean	16.60	13.95	18.17	21.59	18.27	17.71
	a	a	ab	b	ab	

The following data were recorded: Percentage of lodged plants (i.e., plants leaning more than 45 degrees), percentage of broken plants, percentage of barren plants, number of ears per plant, weight of 100 kernels and shelling percentage.

All data were analyzed statistically according to the procedure outlined by SNEDECOR (1956). The letters in the tables are used in accordance with DUNCAN's multiple range test (1955) to compare the treatment means. Any two values within a column not followed by the same letter are significantly different at the 5 per cent level of probability.

1. *Percentage of lodged plants.* Data on the percentage of lodged maize plants as influenced by preceding crops and N fertilizer are shown in Table 1.

Effect of preceding crop. Preceding crop had no significant effect on lodging. It was observed that berseem as preceding crop had good effect in decreasing lodging in maize as compared with other preceding crops, but differences in percentage of lodged plants failed to reach the level of significance.

Effect of nitrogen level. Nitrogen showed a statistically significant effect on lodging. The percentage of lodged plants decreased significantly due to the application of N. The percentages of lodged plants were 18.17, 8.36 and 9.59 for the N levels of 0, 74 and 148 kgs per hectare, respectively.

This result might be due to the effect of N in increasing the stem diameter and root system of maize plants. It is logical that plants having a well developed root system and greater stem diameter can withstand wind and other mechanical effects causing lodging.

Contradictory results were reported by KRANTS—CHANDLER (1951), ZUBER *et al.* (1954), FISHER—SMITH (1957) and HUSSEIN (1968), who found that N increased lodging in maize, while HUSSEIN (1958) found that nitrogen had very little little effect on lodging.

Interaction: preceding crop × nitrogen. The effect of the interaction of preceding crop × N on lodging was not significant.

2. *Percentage of broken plants.* Data on the percentage of broken maize plants, as influenced by preceding crop and N fertilizer are shown in Table 2.

Effect of preceding crop. Preceding crop showed a significant effect on the percentage of broken maize plants. Preceding crops could be arranged in a descending order according to their effect on avoiding breakage in maize as follows: wheat, flax, barley, berseem and field beans, where percentages of broken plants were 13.95, 16.60, 18.17, 18.27 and 21.59, respectively. Wheat and flax were significantly superior to field beans in their effect on decreasing breakage in maize.

Table 3

Mean percentage of barren plants
(Combined analysis of 1969, 1970 and 1971 seasons)

Nitrogen kgs/ha	Preceding winter crop					Mean
	Flax	Wheat	Barley	Beans	Berseem	
0	4.59	3.84	2.94	1.92	0.75	2.81 a
74	0.66	0.73	0.73	1.08	0.11	0.66 b
148	0.50	0.51	0.59	0.35	0.12	0.41 b
Mean	1.92	1.69	1.42	1.12	0.33	1.29
	c	bc	bc	b	a	

Effect of nitrogen level. Nitrogen had no significant effect on the percentage of broken maize plants. This result did not agree with those obtained by HUSSEIN (1958) and HUSSEIN (1968), who found that N increased the breakage of maize.

Interaction: preceding crop × nitrogen. The effect of the interaction between preceding crop and N on the percentage of broken maize plants was not significant.

3. *Percentage of barren plants.* Data on the percentage of barren plants in maize as influenced by preceding crop and N fertilizer are shown in Table 3.

Effect of preceding crop. Preceding crop showed significant effect on the percentage of barren plants. Berseem as preceding crop was significantly superior to all other preceding crops in increasing the fertility of maize plants, while field beans were significantly superior to flax. Differences in percentage of barren plants after wheat, barley and field beans were not significant. Preceding crops could be arranged in a descending order according to their effect in decreasing barren maize plants as follows: berseem, field beans, barley, wheat and flax.

In conclusion, legumes in general, and berseem in particular, had better effect in increasing the fertility of succeeding maize plants, as compared with non-legumes. This result might be due to their good residual effect.

Effect of nitrogen level. Nitrogen had significant effect in decreasing the percentage of barren plants in maize. Percentages of barren plants were 2.81, 0.66 and 0.41, at the N levels of 0, 74 and 148 kgs per hectare, respectively.

In conclusion, N increased the fertility of maize plants. This result is logical since N is essential for the growth and development of the sexual organs of plants, and agrees with those obtained by COLWELL (1947), CARTMILL (1953), HUSSEIN (1958), HUSSEIN (1968) and SHAFSHAK—EL-DEBABY (1971).

Interaction: Preceding crop × nitrogen. The effect of the interaction of preceding crop and N on the percentage of barren plants was not significant.

4. *Number of ears per plant.* Data on the number of ears per plant as influenced by preceding crop and N fertilizer are shown in Table 4.

Table 4

Mean number of ears per plant
(Combined analysis of 1969, 1970 and 1971 seasons)

Nitrogen kgs/ha	Preceding winter crop					Mean
	Flax	Wheat	Barley	Beans	Berseem	
0	0.956	0.973	0.975	1.046	1.075	1.005 a
74	1.019	1.064	1.077	1.161	1.151	1.094 a
148	1.111	1.134	1.119	1.191	1.237	1.158 a
Mean	1.029 a	1.057 a	1.053 a	1.133 a	1.154 a	1.085

Effect of preceding crop. The preceding crop had no significant effect on the number of ears per maize plant. However, the number of ears per plant after legumes was greater than that after non-legumes, but differences failed to reach the level of significance.

Effect of nitrogen level. Nitrogen increased the number of ears per plant, but increases in the number of ears failed to reach the significant level. The number of ears per plant increased by 0.089 and 0.153 over the control due to the application of 74 and 148 kgs per hectare, respectively.

In conclusion, N showed, in general, good effect on the number of ears per plant. However, this result was not statistically evidenced. This result agrees with those obtained by VITTUM (1948), who found that the number of ears per plant did not increase significantly in N treated plots. On the other hand, HUSSEIN (1958), RAI (1961) and SHAFSHAK—EL-DEBABY (1971), found that the number of ears per plant increased significantly due to the application of N.

Interaction: Preceding crop × nitrogen. The effect of the interaction between the preceding crop and N on the number of ears per maize plant was not significant.

5. *Weight of 100-kernels.* Data on the weight of 100-kernels as influenced by the preceding crop and N fertilizer are shown in Table 5.

Effect of preceding crop. The preceding crop showed a significant effect on the weight of 100-kernels of succeeding maize. The weight of 100-kernels after berseem was significantly superior to that after non-legumes, while the 100-kernels weight after field beans was significantly higher than that after flax. Preceding crops could be arranged in a descending order according to their effect on 100-kernels weight as follows: berseem, field beans, wheat, barley and flax. This result might be due to the good residual effect of legumes, and agrees with those obtained by SHORMA—SINGH (1969).

Effect of nitrogen level. Weight of 100-kernels increased significantly as the N level increased. The application of 74 and 148 kgs N per hectare increased the 100-kernel weight by 3.34 and 5.27 grams over the control, respectively. This result agrees with those obtained by HUSSEIN (1958), RAI (1961) and SHORMA—SINGH (1969), whereas KHALIFA (1970) found that differences in 100-kernel weight due to N rates were not significant.

Table 5

Mean weight og 100-kernels, in grams
(Combined analysis of 1969, 1970 and 1971 seasons)

Nitrogen kgs/ha	Preceding winter crop					Mean
	Flax	Wheat	Barley	Beans	Berseem	
0	25.77	26.29	26.15	28.17	30.12	27.30 a
74	29.49	30.89	30.75	30.28	31.80	30.64 b
148	32.09	32.90	31.67	33.26	32.92	32.57 c
Mean	29.12 a	30.06 ab	29.52 ab	30.57 bc	31.61 c	30.17

Table 6

Mean shelling percentage
(Combined analysis of 1969, 1970 and 1971 seasons)

Nitrogen kgs/ha	Preceding winter crop					Mean
	Flax	Wheat	Barley	Beans	Berseem	
0	84.68	85.10	85.43	84.82	85.43	85.09 a
74	85.11	84.33	85.59	84.34	85.63	85.00 a
148	84.40	85.11	85.21	84.70	84.77	84.84 a
Mean	84.73 a	84.85 a	85.41 a	84.62 a	85.28 a	84.98

Interaction: Preceding crop × nitrogen. The effect of the interaction of preceding crop and N on the weight of 100-kernels was not significant.

6. *Shelling percentage.* Data for shelling percentage in maize as influenced by preceding crop and N fertilizer are shown in Table 6.

Effect of preceding crop. The preceding crop had no significant effect on the shelling percentage of succeeding maize. It seems that the shelling percentage is mainly a genetical character, which is not affected by environmental conditions.

Effect of nitrogen level. Nitrogen had no significant effect on the shelling percentage of maize. This result did not agree with that obtained by WOLFE (1924), who found that nitrogen increased the shelling percentage in maize.

Interaction: Preceding crop × nitrogen. The effect of the interaction between preceding crop and N on shelling percentage in maize was not significant.

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SUNFLOWER RESPONSE TO NITROGENOUS FERTILIZATION AT G. R. S.

In 3-year trials, Nicolae (1961) found that sunflower responded to the application of superphosphate only and less favourably to superphosphate + potassium or to complete NPK. Kamal—ElDamiaty (1959) tried three sunflower varieties under four fertilizer treatments and two levels of irrigation. They found that the fertilizer treatments had no effect on the yield, but doubling the amount of irrigation water increased the yield by 40 per cent. In a field trial in Italy, Candussio (1958) reported that the application of NPK increased sunflower seed yields significantly while P had no effect.

This study presents the result of the first attempt to test the performance of sunflower under nitrogenous fertilization and irrigation in G. R. S.; furthermore to evaluate sunflower as a new field crop for Sudan conditions.

The sunflower field trial was conducted at G. R. S., Medani, Sudan, in 1968/69 to 1969/70. The soil is heavy, dark cracking clay with a pH of about 8.5 and a low nitrogen status (about 0.025 per cent). The average annual rainfall was 300 m. m.

The trial consisted of threee sunflower varieties (Armavirec, Arrowhead and Peredovik) and three levels of nitrogen (zero, 45 and 90 kg N per-ha). Sowing was carried out on 1/6 at a spacing of 60 cms between ridges, 20 cms between holes, 3 seeds per hole and thinned to 1 plant per hole after two weeks from planting On the same day of planting the seeds were treated with Aldrex T at 2.2 g per kg of seeds. The fertilizer, Urea, was added at sowing time and as side-dressing. The sunflower crop was irrigated at two weeks intervals and was weeded twice. All the operations from sowing to harvesting were manually conducted. The design of the experiment was randomized block replicated six times and the sub-plot size was 12 × 3.6 m.

Oil content was determined by the laboratory press method (Nur 1973). Hull percentage determination was based on the seed-cutter method (Nur 1969).

The effect of nitrogen fertilizer on the characters tested for the three varieties is shown in Table 1. As expected not all the attributes tested responded to the addition of nitrogen, e.g. hull and oil contents. Although no significant differences were detected between the varieties at each nitrogen level, the magnitude of response differed from one variety to the other, e.g. the addition of 45 kg N per hectare resulted in a seed increase of 13 per cent, 11 per cent and 13 per cent for Armavirec, Arrowhead and Peredovik, respectively. The addition of nitrogen resulted in significantly taller plants, larger head diameter and heavier filled seeds which support the final seed yield obtained. Head diameter is important from the yield point of view as it leads to an increased seed producing area.

Comparing the results achieved in this experiment with other areas producing sunflower we find that the seed yield obtained is markedly poor, a situation which does not favourably encourage raising up sunflower commercially under irrigation in Sudan, as it might not be an economical crop. The other characters which are particularly noteworthy are the great weight of unfilled seeds per head, the poor holding capacity of the seeds in the head, the small head size and the high hull content. It was noticed that the unfilled seeds were located in the centre of the head, this might be due to the fact that the florets away from the periphery of the head are the last to open and because of competition for nutrients, the florets that are the last to open are left with little nutrient supply which result in less developed seeds, consisting mainly of seed coat and pericarp and no cotyledon oil-bearing tissue.

Such a situation suggests that trying sunflower under rain-fed conditions in Sudan, and breeding special varieties with large heads, great seed holding capacity, thin hulled seeds and a greater number and weight of filled seeds per head will be of value.

There is evidence that sunflower responded to fertilizer nitrogen at G. R. S. The addition of nitrogen resulted in greater total seed yield, 1000 seed weight, weight of filled seeds per head, head diameter and plant height. Oil and hull contents were not affected by the application of nitrogen.

The results achieved indicate the limiting factors for the commercial sunflower production under irrigation in Sudan.

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Table 1

Effect of nitrogen fertilizer on some agronomic characters of three sunflower varieties tested at G. R. S.

Variety	Nitrogen level			Variety	Nitrogen level		
	Zero N	45 kg N/ha	90 kg N/ha		Zero N	45 kg N/ha	90 kg N/ha
	Seed yield (kg per ha)				Plant height (cms)		
Armavirec	751	847	953	Armavirec	95.4	99.9	107.8
Arrowhead	840	933	1029	Arrowhead	96.6	101.1	110.2
Peredovik	751	849	941	Peredovik	98.3	102.2	109.5
S.E. ± 12.73		C.V. = 17.1%		S.E. ± 1.02		C.V. = 11.0%	
	Weight of 1000 seeds (g)				*Weight of unfilled seeds/head (g)*		
Armavirec	46.4	52.2	56.4	Armavirec	0.9	0.9	1.1
Arrowhead	48.2	53.0	58.1	Arrowhead	1.5	1.3	1.5
Peredovik	46.1	50.3	55.8	Peredovik	1.1	1.3	1.2
S.E. ± 0.98		C.V. = 8.7%				C.V. = 13.3%	
	Weight of filled mature seeds/head (g)				*Hull percentage*		
Armavirec	11.8	12.5	13.0	Armavirec	26.48	27.24	26.57
Arrowhead	12.2	12.8	13.4	Arrowhead	26.14	26.39	27.42
Peredovik	11.9	12.6	13.1	Peredovik	26.38	27.17	27.69
S.E. ± 0.17		C.V. = 10.2%				C.V. = 5.4%	
	Head's diameter (cms)				*Oil percentage*		
Armavirec	7.4	9.8	12.6	Armavirec	41.01	40.97	40.86
Arrowhead	7.6	9.9	12.8	Arrowhead	40.63	40.94	41.08
Peredovik	7.9	10.3	12.9	Peredovik	42.00	42.06	42.07
S.E. ± 0.74		C.V. = 9.6%				C.V. = 6.2%	

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POTASSIUM SELECTIVITY IN THE SOILS OF EGYPT

Potassium fertilizers are not widely used in Egypt due to the presence of ample amounts of mica and feldspar minerals in the Nile suspended matter. However, the Aswan High Dam has diminished this matter and the application of potassium fertilizers might be necessary in the future.

Many workers have observed that potassium is selectively adsorbed and subsequently fixed by some clay minerals. The close fit of potassium ions within the hexagonal cavities of the 2 : 1 clay minerals was invoked by **Wear—White** (1951) to account for this fixation. More recently, **Rich** (1968) as well as **Sawhney** (1970) concluded that cation hydration energy is more relevant. Cations with low hydration energy such as K, NH_4, and Cs produce interlayer dehydration and layer collapse, thus, they become fixed in the interlayer positions.

Knibbe—Thomas (1972) stated that the 2 : 1 clay minerals show different selectivity for potassium as a function of charge density and its seat on the mineral lattice. Mica minerals are very selective for potassium while montmorillonite is rather non-selective. Yet, **Carson—Dixon** (1972) indicated that some soil montmorillonites have a greater selectivity than many specimen tapes of this mineral. They are of the opinion that the small undetected admixtures of mica and vermiculites in soils might be the reason of such unexpected high selectivity.

The current work was undertaken to study the potassium selectivity of some soils of Egypt. A series of some standard minerals was included in this work for comparison.

Two soil samples were taken from the Experimental Stations of the Faculties of Agriculture at Giza and Zagazig to represent productive soils. A third one was chosen from the highly saline soils of San El-Hagar, Sharkia. The $< 2\ \mu$ fraction of these soils as well as that of montmorillonite (Wyoming bentonite) was separated, saturated with calcium, and its total potassium was determined by the HF—$HClO_4$ method as described in a previous work by **Tahoun—Hamdi** (1973). The clay mineralogical composition of San El-Hagar soil was also investigated by X-ray diffraction.

A biotite specimen (Geology Dept., Ain Shams Univ.) was thoroughly ground in an agate mortar to pass a 200 mesh sieve. The fine fraction was dialysed against a daily renewed solution of 0.1 N $CaCl_2$ for one week to produce a Ca-saturated sample, and four weeks to produce a partially weathered biotite.

For potassium selectivity determinations, the method of **Carson—Dixon** (1972) was used with some modifications. Aliquot containing 0.5 g of biotite and only 0.2 g of each of weathered biotite, montmorillonite, and soils clays was equilibrated for 3 days with graded K—Ca chloride solutions. Total salt concentration was 5×10^{-2} N and K concentration ranged from 5×10^{-4} to 4×10^{-2} N. Thenafter, soluble and exchangeable Ca was determined through

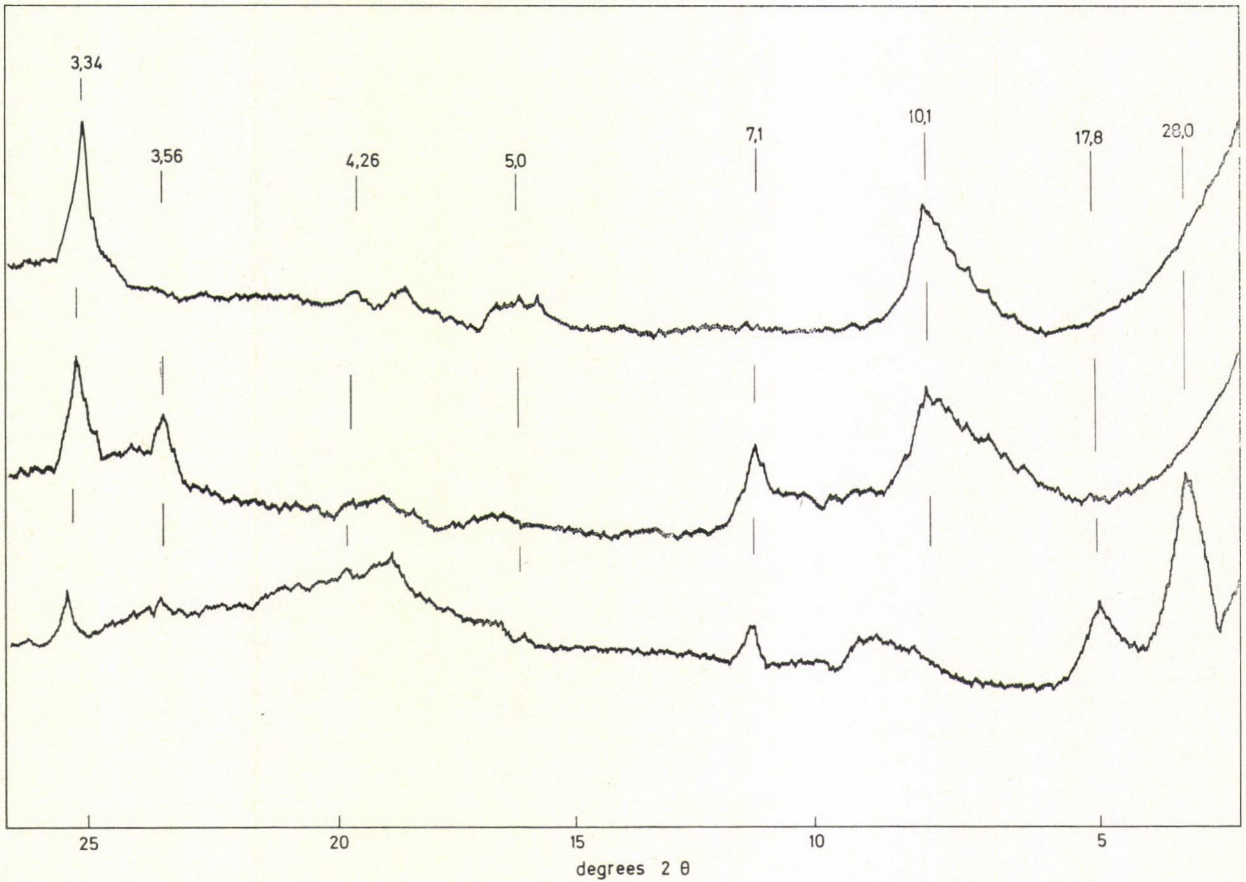

Fig. 1. X-Ray diffraction patterns of the $<2\ \mu$ fraction of San El-Hagar soil with Mg-saturation glycerol solvation (lower pattern), K-saturation and heating at 300 °C (middle pattern), and K-saturation and heating at 550 °C (upper pattern)

titration with EDTA and soluble and exchangeable K was determined by the flamephotometer.

The selectivity coefficient; K_G, was calculated using the Gapon equation:

$$K_G = \frac{{}^{N}K_{ads}}{{}^{N}Ca_{ads}} \cdot \frac{M^{\frac{1}{2}}Ca_{sol}}{MK_{sol}}$$

where N = the meq. of adsorbed K or Ca ions per 100 g clay, and M = the ion concentration in the equilibrium solution in moles L^{-1}.

The X-ray diffraction patterns of the clay fraction of San El-Hagr soil are reproduced in Fig. 1. Dominant clay minerals in this soil seem to include a discrete smectite (18 Å) and

Table 1

Potassium selectivity coefficients of clay minerals and soil clays at different exchangeable K percentages

K %	K_9	K %	K_8
Biotite		Clay of San El-Hagar	
8.5	17.24	10.4	13.36
13.1	11.49	12.3	7.94
18.7	6.10	22.8	5.20
25.5	5.44	32.0	4.51
37.1	4.82	36.2	4.12
56.3	4.83	55.9	4.01
Weathered Biotite		Clay of Giza	
9.2	19.36	9.1	11.45
15.6	14.71	15.6	6.03
22.0	7.06	26.2	3.84
31.4	5.98	35.5	3.98
37.4	6.09	40.9	3.70
55.1	5.86	57.0	3.57
Montmorillonite		Clay of Zagazig	
11.8	1.35	9.8	10.26
15.3	1.42	17.2	5.20
20.3	1.14	25.0	3.29
28.0	1.38	31.3	3.02
36.4	1.29	39.7	3.17
62.5	1.30	61.6	3.05

a regular interstratified smectite-hydrous mica clay (28 Å). A small amount of kaolinite is evidenced by the two peaks at 7.1 and 3.5 Å which disappear upon heating to 550 °C. Quartz is also present in a small amount as indicated by the two peaks at 4.26 and 3.34 Å. This mineralogical composition is similar to that of Giza and Zagazig soils (TAHOUN—HAMDI 1973), but the amount of interstratified clay in these soils is less remarkable.

The potassium selectivity coefficients of clays used in this work were found to be inversely proportional to K-saturation percentage in all cases except montmorillonite as evidenced by the data of Table 1. At medium and high levels of exchangeable potassium, the selectivity coefficients reach a constant value. This result is in a complete agreement with that of CARSON—DIXON (1972), and KNIBBE—THOMAS (1972).

Biotite and weathered biotite give the highest while montmorillonite shows the least selectivity for potassium. This difference could be accounted for by the fact that biotite has a higher charge density than montmorillonite. Also, the interlayers of biotite are frayed at the edges, thus providing the mineral with highly selective sites for potassium as reported by RICH (1968) who found that edge-interlayer sites are much more selective for potassium than interlayer or planar sites. Therefore, it should be expected, according to SAWHNEY (1970) that a partially weathered biotite would be more selective for potassium than biotite as it contains a greater number of edge-interlayer sites. The data of Table 1 for biotite and partially weathered biotite are consistent with this expectation.

Within the soil clays under investigation, the data of Table 1 indicate that the soil of San El-Hagar is the most selective. Moreover, the selectivity values of San El-Hagar, Giza, and Zahazig clays correlate very closely with their hydrous mica contents which amount to 20, 15, and 12 per cent, respectively. A similar relation was reported by CARSON—DIXON (1972) in some montmorillonitic soils.

The fairly high selectivity of the clay fraction of San El-Hagar soil could not be interpreted only by its hydrous mica content, since the pronounced influence of this mineral is likely to be observed only at low K percentages. Probably, it could be explained by the nature of the existing smectite. TAHOUN—HAMDI (1973) have established that the hydrous mica of the alluvial soils of Egypt belongs to the muscovite series of mica. Consequently, it could be assumed that the original muscovite in San El-Hagar soil might have been attacked by the excessive salts in the soil, thus it was transformed into a smectite mineral; biedellite. This mineral with its charge on the tetrahedral layer, was reported by WEAR—WHITE (1951) to offer a closer distance between the lattice negative and potassium ions than montmorillonite whose charge is located on its octahedral layer. Moreover, FARMER—RUSSELL (1967) added that the negative charge of biedellite is more localized, and therefore, it would be more selective for potassium compared with montmorillonite.

Acknowledgement

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*

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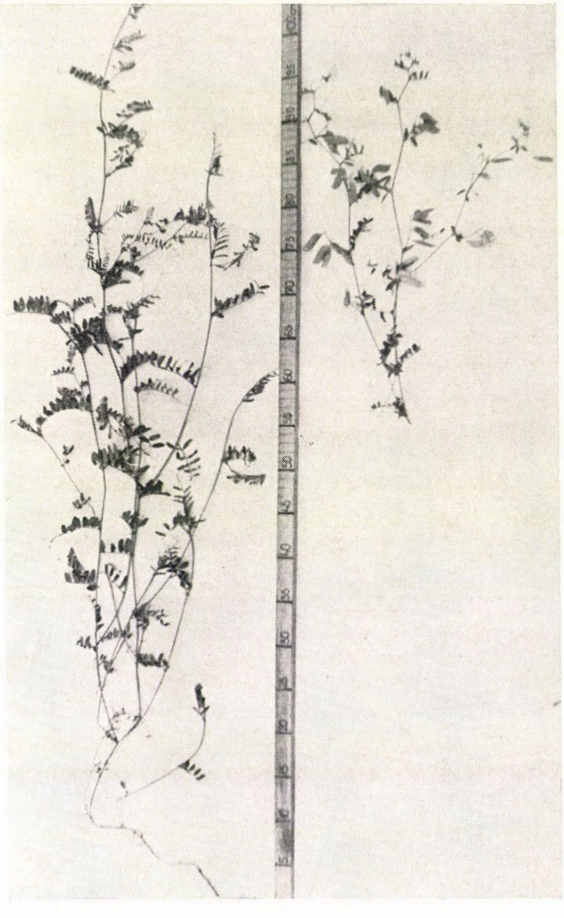

"F" VETCH

Taxonomical place: *Vicia villosa* Roth ssp. *pseudovillosa* (Schur) J. Murr.
Origin: selected from a wild population and improved by family breeding.

Beginning of breeding: 1928, Kompolt.

Breeder: †Fleischmann, R. (Kompolt); variety maintainer Bócsa, I. (Kompolt).

State qualification: provisionally certified variety 1954, state certified variety 1967.

General characterization: with its almost hairless shoots it is easily distinguished from the main type of hairy vetch; a valuable winter-hardy fodder plant with satisfactory yields in moister sandy soils too.

Morphological description:

Root-system: the primary root penetrates 80—120 cm deep into the soil, on the secondary roots abundantly branching from the primary root there are patulous root-nodules.

Shoot-system: its 60—150 cm long branching main shoot is either procumbent or — when combined with rye — climbing.

Stem: thin, fine, almost hairless.

Foliage: even-pinnate leaf, compound ending in a long tendril. On the rachis there are 8—10 pairs of lanceolate-ovate, finely shaped, thinly haired, entire leaflets with bluntly pointed tips.

Inflorescence: medium large cluster with 20—30 flowers.

Flower: 12—20 mm long; the calyx is of dark yellowish green colour with uneven teeth; the corolla varies from purple to light blue; the anthers are yellow and of oblong egg-shape.

Fruit: 20—40 mm long, 5—8 mm wide, light or dark brown flat pod with 4—7 seeds.

Seed: regular sphere of 3.5—4 mm diameter; glabrous, mat; of dark bluish-grey (almost black) colour with a dark brown hilum. Thousand-grain-weight 25—30 g, hl-weight 81—83 kg (KAPÁS *et al.* 1965, PÓSA 1970).

Biological characters:

Germination: cardinal points: minimum + 3 °C, optimum + 25 °C, maximum +35 °C (JÁNOSSY *et al.* 1971). The extent of hard-coatedness may even be 30 per cent.

Vegetation period: ripens at the same time as the unimproved hairy vetch; flowering begins in the second half of May and the first pod ripens a month later (PÓSA 1970).

Winter hardiness: excellent

Resistance to diseases: not susceptible

Farm technology requirements:

Sowing: optimum time in the middle of October

Soil requirement: with the exception of dry sandy soils it develops well in soils suitable for the crop sown with it.

Production: seed yield 1—7 q/ha; green yield together with the joint crop 150—250 q/ha (JÁNOSSY *et al.* 1971).

Area of cultivation: yields sufficiently on the entire area of Hungary.

*

Prepared at the Department of Botany, University of Agricultural Sciences, Debrecen

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FORUM

DICHOTOMOUS BRANCHING OF VASCULAR BUNDLES IN THE STEM AND LEAF OF MAIZE AND THEIR PHYLOGENETIC IMPORTANCE

In her candidate's dissertation "Effect of some ecological factors on the anatomy of the vegetative organs of the maize stem" (1973) S. **Volcsánszky** dealt — among others — with the number of vascular bundles in the stem and leaves of maize and their changes in some nodes in detail. She pointed out: "twin bundles are frequent at all levels of the axis", and "adjacent bundles grow together vertically". The author began counting the internodes from the top and continued downward to the roots. If, however, she had counted them from the base upward to the tassel, the above sentence would have been modified in the following way: "Twin bundles are frequent at all levels of the axis", "the adjacent bundles separate vertically". The author presented a figure and a table where an increasing number of vascular bundles found in the successive internodes was indicated (Table 1). Accordingly, the number of vascular bundles decreases from the base upward.

Greguss had a closer look at Volcsánszky's data and immediately suspected a dichotomous branching of the bundles in the nodes, because the vascular bundles running into the leaves and those entering the internodes were almost of equal number, that is fifty-fifty per cent. In his opinion the vascular bundles running upward separate dichotomously in the nodes, or at the bases of the leaf-sheaths, half of them branching off into the leaves, the other half continuing their way in the stem. Thus the appearance of twin bundles ought to be interpreted this way. And if so, it has — according to Greguss — a phylogenetic importance. Volcsánszky did not discuss such phylogenetic aspects in her dissertation — which was not the aim of her work — still she established facts from which important and interesting phylogenetic conclusions could be drawn. What are these facts? From Volcsánszky's data — determined by the precise examination of a number of maize plants — only those concerning the number of vascular bundles counted in the internodes (plus some photos) were used by Greguss who found certain regularities in them.

Table 1 shows that the changes in the number of vascular bundles are almost uniform in the different organs of the maize shoot and at different heights of the stem. For the sake of a better understanding all this is presented in a figure too (Fig. 1). In Volcsánszky's drawing the internodes — 13 in number — are numbered from the top downward. The first internode bears the tassel, while the lowest — 13th — level is near to the adventitious roots. She could have begun numbering from the base, but that makes no difference; the number of the vascular bundles does not change whether we begin counting them from above downwards, or from below upwards. In the annexed table we too began numbering the 13 internodes at the top.

In the second column marked with *a* the number of vascular bundles running in the internodes is shown on the basis of Volcsánszky's investigations. They are true numbers and our further investigations are primarily based on them. Considering the order of magnitude and succession of the numbers the vascular bundles of the internodes were supposed to branch off dichotomously in the node above, where their number theoretically redoubled. This column is marked with *b*, where the value of $b = 2a$ means 100 per cent of the existing vascular bundles.

Table 1

Number of vascular bundles in the nodes and internodes

Internodes	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th
Number of vascular bundles in the internodes (Volcsánszky's data)	(a) 173	222	264	283	364	373	429	451	451	442	401	398	385
According to Greguss after the dichotomous branching of the ascending stem bundle the vascular bundles may redouble in the nodes. This number is the theoretical maximum 100 per cent	(b=2a) —	444	528	566	728	746	858	902	902	884	802	796	770
By subtracting the actual number of vascular bundles in the internodes from their theoretical number in the nodes we obtain the number of bundles running into the leaf sheaths and laterals	(c=b—a) 72	306	302	445	382	485	473	451	433	360	395	372	
			(200—400)				(500—900)			(150—300)			
The ratio of the actual number of bundles in the internodes to their theoretical number in the nodes gives the percentage of the stem bundles	$(d=\frac{a}{b}\%)$	42	46	38	49	43	46	50	50	55	50	51	
The ratio of the bundles entering the leaf sheaths and laterals (c) to the theoretical number of bundles (in the nodes (b) gives the percentage of bundles in the leaf sheaths and laterals	$(e=\frac{c}{b}\%)$	58	54	62	51	57	54	50	49	45	49	49	

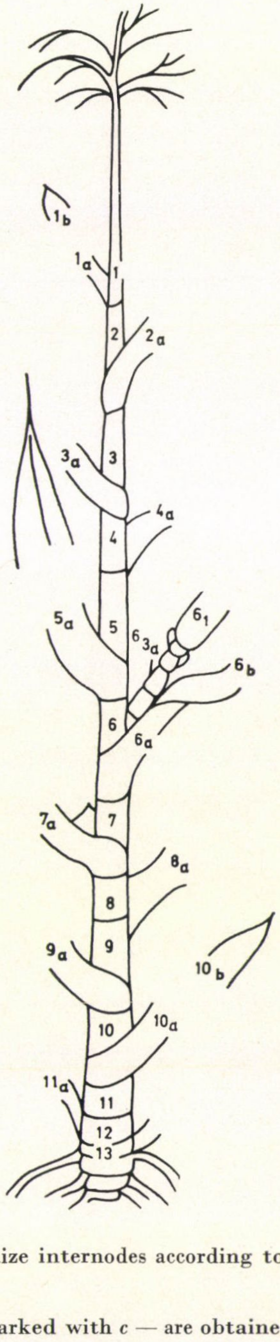


Fig. 1. Numbering of maize internodes according to S. Volcsánszky

The numbers in column 3 — marked with *c* — are obtained by subtracting the number vascular bundles found in the internodes from the total number, that is: $c = b - a$. This latter number shows, in turn, that the other half of the vascular bundles branching off dichotomously in the nodes continue in the leaf blades. The figures found in brackets after these numbers show

the number of vascular bundles actually observed by Volcsánszky to run into the leaves. These figures prove, in addition, that the theoretically determined and actually observed data are almost identical, demonstrating that most of the vascular bundles of the internodes really branch off dichotomously in the nodes.

In the next column (*d*) the percentages of the above data are presented; they show the percentage ratio of the number of vascular bundles actually counted in the internodes to those theoretically calculated and dichotomously branching in the nodes. Its formula is: $d = a/b$.

The figures of the last column show the ratio of the number of bundles entering the leaves to the theoretically supposed 100 per cent number of bundles in the nodes. From the last two columns it can be established on the whole that the two percentage ratios more or less agree, that is, the dichotomous branching of the vascular bundles in the nodes of the maize plant is highly probable.

Let us see the other proofs. According to Volcsánszky's data in the lowest, i.e. 13th internode there were 385 vascular bundles. If we suppose a dichotomous branching of each of them in the next node, then the number of bundles ought to be twice as much as before, that is 770. In the next, i.e. 12th internode 398 actual vascular bundles run upwards, while the rest, i.e. the theoretical 372 branch off towards the leaves. The former is 51 while the latter 49 per cent of the total number of bundles in the node, that is, they are almost equal. However, the bundles of the 12th internode advance to the next node, where — if each of them branches dichotomously — a maximum of 796 bundles may be produced. However, only 401 of them entered the next internode, while 395 were left to the leaves. The number of bundles advancing to the internode is, in this case too, 51 per cent, while that of the bundles entering the leaves 49 per cent. The situation is similar in the 11th internode too. From the 401 bundles counted here 802 may be produced in the next node if each of the bundles branches dichotomously. Volcsánszky counted 442 in the 10th internode which was 55 per cent of the total, and the remaining 360, i.e. 45 per cent of the total, must have entered the leaves.

The further fate of the vascular bundles of internodes 10 and 9 is especially interesting. The 442 bundles of the internode may theoretically be separated into 884 dichotomous branches in the 9th node. Of this in the next internode 451 bundles were actually found, and 433 may have run into the leaves, which means 51 and 49 per cent, respectively.

All this is best shown, however, by the 9th and 8th internodes. The 451 bundles of the 9th internode can theoretically be divided into 902 bundles. The 451 stalk bundles actually counted in the 6th internode is equal to the number of bundles possibly entering the leaves, that is the ratio here is exactly fifty–fifty per cent.

We may continue these calculations up to the second and first internodes. In internode 2 the actual number of vascular bundles was 222, which — according to the theory — could only divide into 444 bundles in the first node. Of this the first internode was entered by 173 bundles, while the tiny leaves were only supplied with 72, according to the data. This remarkably small number may be due to the stem becoming thinner, and to the lower number of vascular bundles in the first tiny leaf. Nevertheless, according to Volcsánszky's data 200—400 vascular bundles may even occur in the uppermost leaves, which perfectly agrees with the theory

To sum up the results, it can be established that in the lowermost internodes of the maize stalk the number of vascular bundles is somewhat lower than in those upwards from internode 11. The largest number of vascular bundles (451) is found in the 7th internode, where generally the largest leaves and lateral shoots develop; from this up to the tassel the vascular bundles gradually decrease in number.

As to the percentage proportion of the vascular bundles branching off into the leaves, these values are largely about 50 per cent in all cases. And this seems to confirm the dichotomous branching of the vascular bundles in the nodes. Smaller or greater deviations from this proportion may, naturally, occur. Namely, not all vascular bundles running upwards branch in the next node, some of them sometimes pass through several nodes and only branch in the 4th or 6th one. These are the major bundles. All this is seen in the figures of Plates I and II.

Fig. 1 of Plate I shows, e.g. the cross-section of a twin bundle, making it perfectly clear that it is a case of separation and not of growing together. Fig. 2 shows again the cross-section of a minor bundle; both are collateral closed bundles. Fig. 3 is a photo taken of the root zone; both are well defined amphivasal bundles. Figs 4, 5 and 6, on the other hand, show the longitudinal section of the twin bundles. The arrows clearly show that twin bundles are always formed around the nodes. Fig. 7, on the other hand, is an example of some bundles passing sometimes through even 4 or 5 nodes without branching. These are the so-called major bundles.

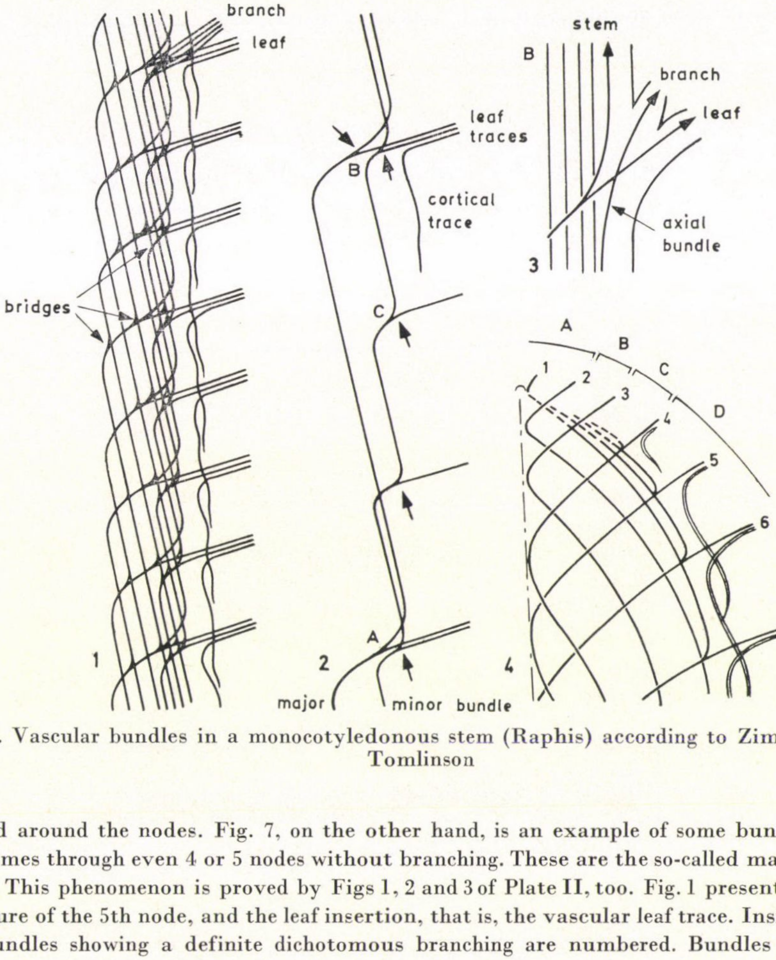


Fig. 2. Vascular bundles in a monocotyledonous stem (Raphis) according to Zimmermann—Tomlinson

This phenomenon is proved by Figs 1, 2 and 3 of Plate II, too. Fig. 1 presents the radial structure of the 5th node, and the leaf insertion, that is, the vascular leaf trace. Inside the stem the bundles showing a definite dichotomous branching are numbered. Bundles Nos 1 to 6 show not only a dichotomous branching, but also an almost regular arrangement from the medulla to the cortex. In the same way, bundles Nos 8—15 gradually advance from the cortical zone to the periphery of the stem, then continue in the leaf-sheath, clearly showing that the bundles run into the leaf-sheath, too. In the centre of the stem the simple bundles are relatively thinly, while towards the periphery more thickly set, still they show a certain order of succession. Fig. 2 shows a slightly magnified detail of Fig. 1, where the dichotomous branching of the vascular bundles is even more apparent.

The question now arises whether all these have any phylogenetic significance as referred to in the title. They certainly have. Dichotomous branching is still a rather frequent phenomenon in monocotyledonous plants, and this applies not only to the crotches of the big monocotyledonous trees (See Plate III, palms, *Liliaceae*, etc.) but also to the dichotomous branching of the root-hairs of some grasses, e.g. *Triticum.* The parallel leaf veins of the monocotyledons too can, in essentials, be traced back to dichotomy, as one of the oldest forms of branching, which appears in the stem and leaves of maize, too.

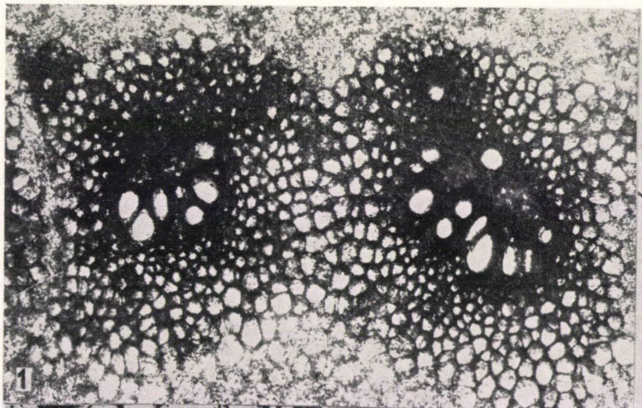
Anyway, dichotomy has always been, and is even today present in the terrestrial flora from the simplest form to the highest phanerogamous plants, and that not only in the stems and trunks, but also in the leaf veins. In the course of phylogenesis dichotomy began already in the simplest filamentous algae, *Chlorophyceae* (e.g. *Vaucheria*), continued in the *Bryophyta (Hepaticae)*, the *Psilophyta (Protopteridium)*, the *Pteridophyta (Pteropsida, Sigillaria, Isoetes)*, the *Pteridospermae (Lyginodendron)*, the *Gymnospermae (Cycadales)*, the *Chlamydospermae (Welwitschia)* up to the *Angiospermae*, so in the *Monocotyledones* and — as we can see — in the vascular bundles of maize, too. Therefore, the dichotomous branching of the vascular bundles in the maize stem can be regarded as a phylogenetic character, some sort of reliquiae.

In connection with dichotomy another thought arises. Namely, in the world of plants two other forms of branching exist besides dichotomy: monopodial and verticillate branching. Monopodial branching — like dichotomy — has always been present both in the simplest filamentous algae and in the most developed angiospermous flowering plants, so in the *Dicotyledones*, too. The most interesting point of this phenomenon is that these three forms of branching have appeared in the course of phylogenesis, and appear even today, always side by side, at the same time and at the same stages. So, e.g. in the monopodially branching green algae, *Chlorophyceae (Cladophora)*, the *Bryophyta (Musci)*, the *Psilophyta (Rhynia)*, the *Pteridophyta (Lycopsida, Lepidodendron)*, the *Pteridospermae (Lepidocarpon)*, the *Chlamydospermae* (Gnetum) and the *Angiospermae*, more closely: in the *Dicotyledones.* And if it is so, then the monocotyledons which show a tendency to dichotomy could not originate from the monopodial dicotyledons — as supposed by some authors —, since in the present dicotyledons, first of all arboraceous dicotyledons true dichotomy does not exist, so they could not transmit such characters to the monocotyledons. Accordingly, the monocotyledons could not originate from the dicotyledons. Namely, in our opinion the monocotyledons have developed to their present stage independently of the dicotyledons. Consequently, the development of the Angiospermae must have been polyphyletic rather than monophyletic. The above results of investigations provide further evidences of the correctness of this theory.

*

Plate I

Fig. 1. Cross-section of a twin bundle at the place of branching (100×)
Fig. 2. Twin bundle from the cortical part of the stem (120×)
Fig. 3. Amphivasal twin bundle from the root zone (120×)
Fig. 4. Places of origin of twin bundles in the trunk of a monocotyledonous palm-tree (Raphis), and their pathway in the leaves and the stem. The points indicate the places of the vascular bundles in sections made at every 5 mm, and the lines connecting them show the direction of advance and branching of the individual bundles. (Tomlinson and Zimmermann's work). The branching of the vascular bundles is dichotomous as in the maize
Fig. 5. Major and minor twin bundles in a monocotyledonous palm stem (Raphis). The arrows show the places of dichotomous branching (Tomlinson—Zimmermann)
Fig. 6. At the white marks the two "Ascending" bundles show a definite dichotomous branching (30×)
Fig. 7. Longitudinal section of a young (5 weeks old) maize stalk. Some bundles pass through 3—5 nodes without branching. These are the "major bundles". The arrow shows the beginning of the dichotomous branching (5×). 1, 2, 3, 6 and 7 are from Volcsánszky's paper



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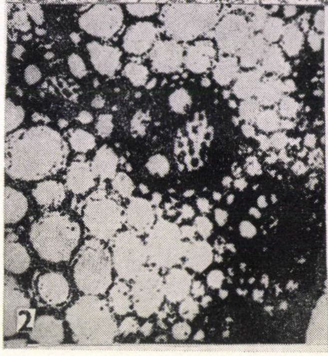

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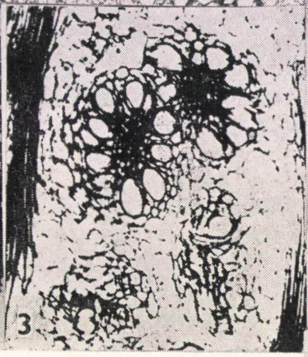

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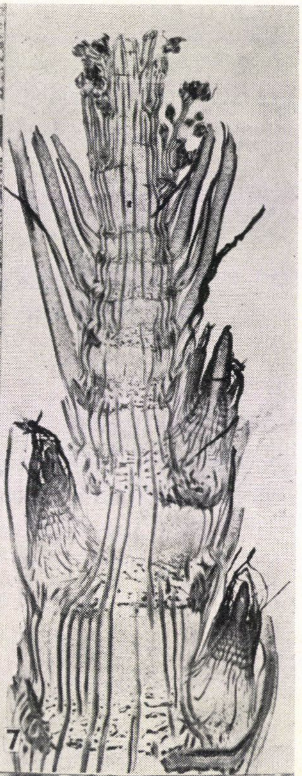

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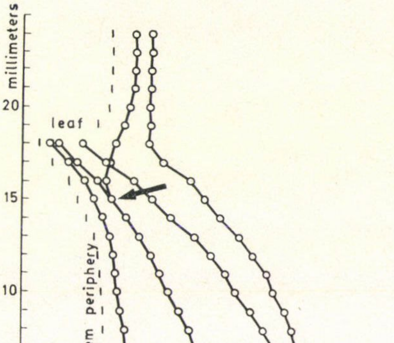
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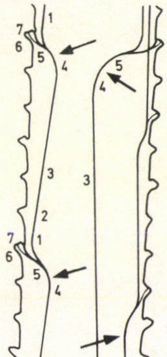
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After having completed and sent to the editorial office the above paper I received a very interesting work published in 1972 by Martin H. Zimmermann and P. B. Tomlinson, professors at the Harward University: "The vascular system of monocotyledonous stems". This paper discusses in detail the problem of vascular supply to the different parts of monocotyledonous plants. The authors took the widely accepted ideas expressed by Desfontaines in 1798 and by Hugo von Mohl in 1824—58 concerning the vascular structure of palms into consideration. On the basis of several years of investigations they revised and modified these old theories. As to the results of their investigations seven drawings are also presented in addition to the detailed descriptions. The drawings and explanations of this paper greatly support the statements made in my paper. I present here a small detail of the text and four figures.

"Figure 2 (1) shows in an idealized and simplified way the monocotyledonous vascular system of a mature vegetative stem in a radial plane. The diagram represents the principles which were first found in *Raphis (Palmae)* and *Prionium (Juncaceae)* (ZIMMERMANN—TOMLINSON 1965, 1972) but subsequently also in other species in several families. Eight leaf insertions are shown, the vascular supply to each leaf represented by only three bundles — two vascular leaf traces (one major, one minor) and a fibrous cortical trace. These three elements, which essentially make up the vascular structure of the stem, are shown separately in Figure 2. In an actual stem, the total number of bundles continuous into a leaf is of the order of hundreds, and there is a gradation from those which originate centrally to those which originate peripherally. This large number of traces is accomodated in a broad leaf insertion which, in most monocotyledons, completely encircles the stem". The main point here is that these bundles continue in the leaf.

Fig. 2 shows, further, that in the stem there is a so-called major bundle which passes through 3—4 nodes and branches off into the leaf only then, while the other branch proceeds in the stem. It was mentioned in my paper too, but I did not know then that it had a separate name. The minor bundle running beside it generally branches off in every node. The main point is that one branch of the dichotomous bundles enters the leaves, while the other continues in the stem.

Section 3 of Fig. 2 clearly shows that it is in the nodes that the vascular bundles branch off, and that one of the branches continues in the leaf, while the other in the stem.

Section 4 of Fig. 2 shows the longitudinal section of the growing tip of a palm trunk. Even the primordial vascular bundles display a dichotomous branching.

Since in the phylogenesis of the flora of the earth dichotomy has always existed in various types of plants, the dichotomous structure of the vascular bundles of the maize plant is considered by the author to be of phylogenetic importance.

Plate II

Fig. 1. It is a magnified detail of the picture in the middle. Numbers 2—6 indicate the dichotomously branching bundles (cca 8×)

Fig. 2. Cross-section of node 5 in a young stem. The thick line shows the succession of the dichotomously branching bundles in the nodes (1—6 and 8—15). In the inner part dichotomous branching is seen in more than one places (5×)

Fig. 3. Magnified detail from the middle picture. The dichotomous bundles → proceed at identical distances from the inner part of the stem towards the cortex and the leaf sheath, respectively (6×). The cross sections of twin bundles in the leaf sheath are connected by dotted lines


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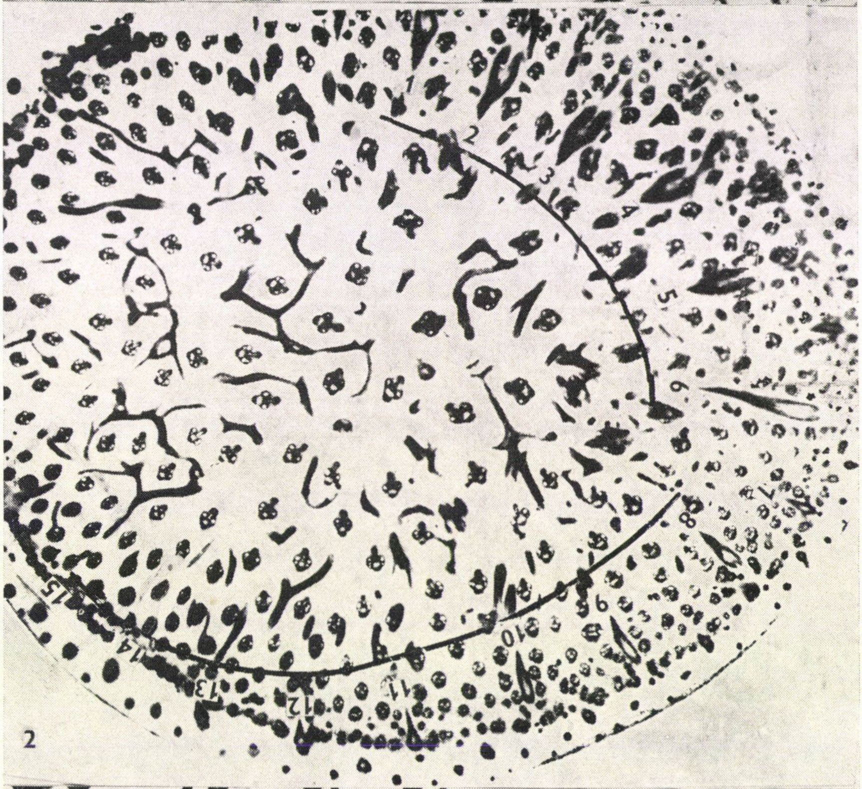
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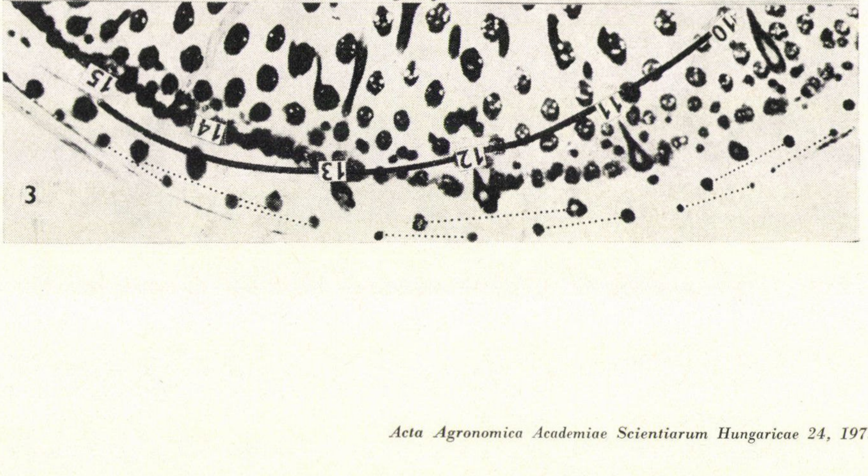
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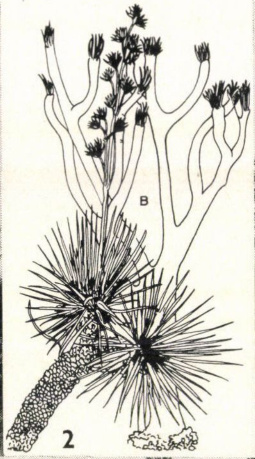

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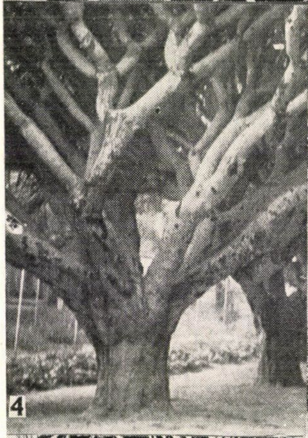

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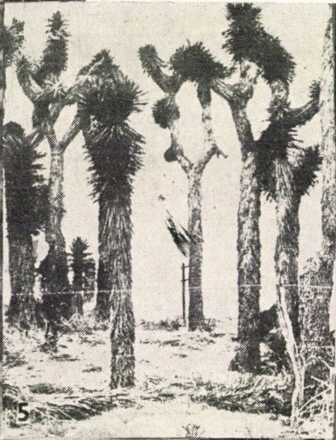

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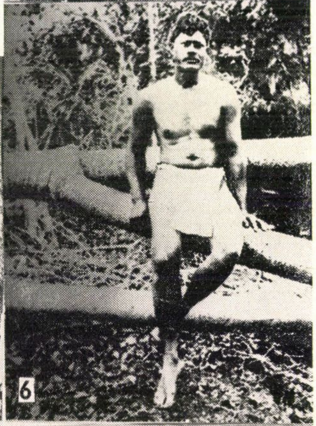

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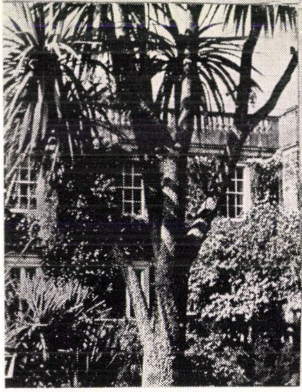

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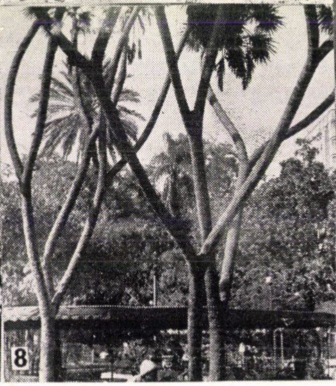

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Acknowledgement

The author is indebted to S. Volcsánszky E., candidate for placing her valuable investigation results and some microphotos at his disposal, because without them the above important results could not be obtained.

*

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Plate III

Fig. 1. Dracaena furcata stem showing a multiple dichotomous branching. Kew Garden, London (Author's photo)

Fig. 2. Dichotomous branching of *Microdracoides squamosus*, an about 1 m high sedge in South-Africa

Fig. 3. Dichotomous branching of a palm from Cuba

Fig. 4. Multiple dichotomous branching of a *Dracaena draco* trunk in a street of Algir (Author's photo)

Fig. 5. Dichotomous branching of a *Yucca arborescens* trunk. Central-America

Fig. 6. Dichotomous branching of a *Pandanus tectorius* trunk. Hawaiian Islands

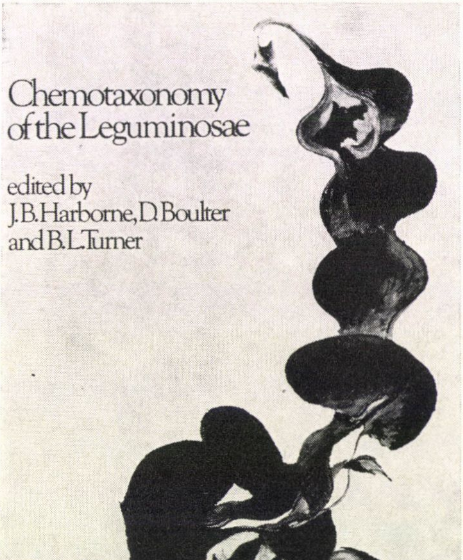
Fig. 7. Dichotomous branching of a *Yucca* trunk. North-Ireland, Park (Author's photo)

Fig. 8. Multiple dichotomous branching of a *Hyphaena thebaica* trunk. Cairo Park (Author's photo)

Fig. 9. Dichotomous branching of an *Aloe beddomei* trunk. Munich. Botanical Garden (Author's photo)

Acta Agronomica Academiae Scientiarum Hungaricae Tomus 24 (3–4), pp. 485–490 (1975)

RECENSIONES



J. B. Harborne, D. Boulter, B. L. Turner: *Chemotaxonomy of the Leguminosae*. Academic Press, London and New York, 1971.

The complete chemotaxonomic processing of the family *Leguminosae* was published as the joint work of 19 well-known experts. The monography of 612 pages extent is made clearer and easier to survey by numerous structural formulae and figures and tables Each of the 15 chapters is completed with detailed literary references. Beside *Hegnauer*'s serie of "Chemotaxonomie der Pflanzen" (Birkhäuser, Basel—Stuttgart) this work is the most up-to-date plant chemotaxonomic monography. Its significance is increased by the fact that the family *Leguminosae* is one of the most important families of angiosperms which, besides the family *Gramineae*, includes cultivated plants of the highest economic value.

With the knowledge of chemotaxonomic correlations based on biochemistry the taxonomy of the family *Leguminosae* makes the phylogenetic problems clearer. In the monography not only the well-known low molecular weight compounds (flavonoids, alkaloids, non-protein amino acids, sugars, lipids and terpenoids) but also the macromolecular components (albumins, globulins and polysaccharides) are discussed, moreover, separate chapters deal with the enzymes and phytohaemagglutinins which are increasing in importance from genetic and resistance biological points of view. It has to be underlined that the proteins and amino acids, highly important from an economic point of view, are discussed with full particulars.

The monography consists of the following chapters:

Chapter 1. The *Leguminosae* — A systematic purview (V. H. Heywood). Summarizes and characterizes the genera belonging to the family *Leguminosae*. Its major parts are: taxonomic divisions; The *Mimosoideae;* The *Caesalpinioideae;* The *Swartzieae;* The

Lotoideae (Papilionoideae); Origin, relationships and evolutionary trends.

Chapter 2. Distribution of flavonoids in the *Leguminosae* (J. B. Harborne). Flavonoid patterns in the *Leguminosae:* anthocyanins; flavonols and flavones; chalcones and aurones; leucoanthocyanidins and flavanones; isoflavonoids and neoflavonoids. Flavonoid surveys at the generic level: general considerations; flavonoids of the genus *Baptisia;* Legume heartwood surveys; Surveys of fodder crops; Surveys in the *Vicieae;* Surveys in the *Genisteae.*

Chapter 3. Alkaloids in the *Leguminosae* (James A. Mears). Approaches to the application of alkaloid data for plant systematics (Variation in alkaloid content for individual plants and for populations; Variation in the alkaloid content of plants during development; Variation in the alkaloid content in populations resulting from hybridization and introgression); Systematically significant alkaloids in the *Leguminosae* (Quinolizidine (Lupine) alkaloids; Pyrrolizidine alkaloids; Phenylalanine- and Tyrozine-derived alkaloids; Tryptophane-derived alkaloids; Physostigma alkaloids; Erythrina alkaloids; Ammodendrine-Hystrine alkaloids; Erythrophleum alkaloids; Smirnovine alkaloids); The structures and distribution of alkaloids in the *Leguminosae.*

Chapter 4. Comparative biochemistry of non-protein amino acids (E. A. Bell). Potential value of amino acids in comparative studies; The possible origins and biological significance of "uncommon" amino acids (Possible origins; Possible biological significance); Amino acids of the *Lotoideae* (Canavanine; Amino acids of the tribe *Phaseoleae;* Amino acids of *Lathyrus* and *Vicia;* Amino acids of *Astragalus*); Amino acids of the *Mimosoideae* (Amino acids of *Acacia* and *Schrankia*)*;* Amino acids of undetermined distribution.

Chapter 5. Distribution of monosaccharides, oligosaccharides and polyols (J. E. Courtois and F. Percheron). Monosaccharides; Oligosaccharides; Cyclitols (myo-Inositol, Quercitol, Pinitol); Metabolism of monosaccharides, oligosaccharides and cyclitols.

Chapter 6. Lipids of the *Leguminosae* (I. A. Wolff and W. F. Kwolek). Seed oil content; Total unsaturation in seed oils; Fatty acid composition of seed lipids; Component glycerides of seed oils; Nonglyceride seed oil constituents; Lipids of plant components other than seeds.

Chapter 7. Terpenoid and other low molecular weight substances of systematic interest in the *Leguminosae* (J. B. Harborne). Simple phenolics; Quinones; Terpenoids (diterpenes, triterpenoids, carotenoids); Growth regulators; Phytoalexins; Miscellaneous constituents (nitrogen compounds; some carbon compounds).

Chapter 8. Taxonomic aspects of the structure of legume proeins (D. Boulter). General characteristics (structure; biosynthesis; function; classification); Phylogenetic and taxonomic considerations (methods applicable to the establishment of the phylogenetic relationships of major plant groups); Methods applicable generally.

Chapter 9. Serology of the *Leguminosae* (J. Kloz). *Sophoreae; Podalyrieae; Laburneae; Cajaneae; Phaseoleae; Glycineae; Vicieae; Trifolieae; Loteae; Stylosantheae.* (Immunelectrophoregrams of major protein types are also presented.)

Chapter 10. Phytohaemagglutinins (G. C. Toms and Ann Western). Blood group specific phytohaemagglutinins; Non-specific, multi-specific and other phytohaemagglutinins; Plant mitogens; Chemistry of phytohaemagglutinins; Botanical aspects.

Chapter 11. *Comparative studies of legume enzymes* (D. A. Thurman). Potential usefulness of enzyme types in systematics; Methods of comparing enzyme structure; Effects of extraction and plant material on detection of enzymic activities; Enzyme types (aminoacyl-sRNA synthetases; lysine biosynthesis; proteinases; dehydrogenases; esterases; fructose-1,6-diphosphate aldolases); Comparisons of amino acid sequences of legume proteins (pea embryo histone IV; ferredoxins).

Chapter 12. *Urease, a typical seed protein of the Leguminosae* (C. J. Bailey and D. Boulter). Properties of Jack bean urease;

Comparison of Jack bean urease with urease from other sources; Function of urease; The structure of urease and its relationship to seed physiology; Regulation of enzyme synthesis and activity; Distribution of urease within the *Leguminosae.*

Chapter 13. *Polysaccharides in the Leguminosae* (R. W. BAILEY). Structural polysaccharides (pectic substances; non-cellulose, hemicellulose polysaccharides; cellulose); Reserve polysaccharides (starch; seed galactomannans; seed "amyloid"; miscellaneous).

Chapter 14. *The amino acid sequence of Phaseolus aureus (Mung-bean) cytochrome c with reference to phylogeny* (D. BOULTER and E. W. THOMPSON).

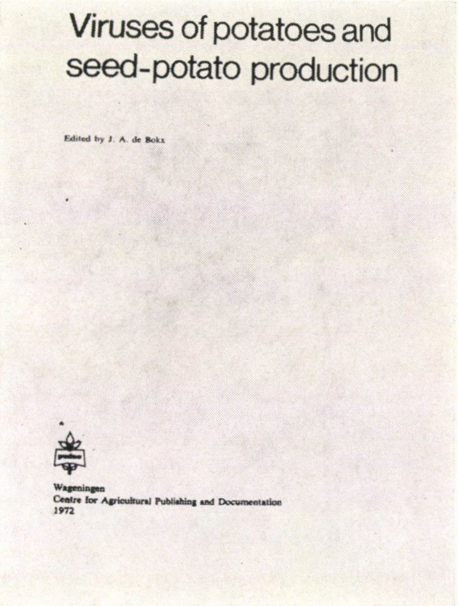
Chapter 15. *Implications of the biochemical data: a summing up* (B. L. TURNER).

The valuable monography summing up the comparative taxonomy, chemistry and biochemistry of *Leguminosae* gives a substantial help to experts in all fields of agriculture; botanists, biochemists, physiologists, specialists of organic chemistry and food chemistry, those performing quality control, pharmacologists, animal breeders, etc., since the full knowledge of this plant family containing many valuable high protein content sources is — especially in these days — indispensable for the efficient solution of world food problems.

L. GY. SZABÓ

J. A. DE BOKX (ed.): *Viruses of potatoes and seed-potato production.* PUDOC, Wageningen, 1972.

The increasing spread of potato viruses and the seed-potato production problems involved set the experts dealing with theoretical and practical questions more and more serious tasks in all potato growing countries of the world. It is for this reason that the book "Viruses of potatoes and seed-potato production" which supplied a great want in the special literature is especially remarkable. The work of a Dutch collective of fourteen authors participating in the research of potato viruses and the production of seed-potato, and highly familiar with both theoretical and practical problems in this field the book deals with the most fundamental questions a knowledge of which is today indispensable when growing healthy seed-potatoes.


Viruses of potatoes and seed-potato production

Edited by J. A. de Bokx

Wageningen
Centre for Agricultural Publishing and Documentation
1972

The book contains 233 pages including 66 figures, 8 coloured pictures and 18 tables. It is divided into 17 chapters with references at the end of each chapter. Chapter 1. (J-P. H. VAN DER WANT: *Introduction to plant virology*) gives a historical survey of potato viruses and describes their major characteristics. Chapter 2. (J. A. DE BOKX: *Graft and mechanical transmission*) deals with the hygiene and methodology of virus transmission. Chapter 3. (D. HILLE RIS LAMBERS: *Aphids: their life cycles and their role as virus vectors*) discusses the population conditions of the major aphid species playing a role in virus transmission, and some questions of the biological control of vectors. Chapter 4. (H. A. VAN HOOF: *Soilborne viruses*) deals with viruses transmissible by fungi and nematodes. In this chapter a short information is given on the control of soil-borne viruses.

Chapter 5. (D. Z. Maat: *Virus purification*) gives an up-to-date view of the possibilities for virus purification by describing the most fundamental methods. Chapter 6. (J. A. de Bokx: *Electron-microscopy*) presents the electron-microscopic methods and diagnostic problems. This chapter is closely connected with Chapter 7. (D. H. M. van Slogteren: *Serology*) which discusses the questions of serological virus diagnosis. Chapter 8. of the book (J. A. de Bokx: *Test plants*) describes the methods of inoculation, the factors influencing the susceptibility of the host plants and presents the local and systemic host plants. Diagnostic questions are dealt with in Chapter 9 too (J. A. de Bokx: *Histological, cytological and biochemical methods*). In Chapter 10, the largest chapter of the book (A. B. R. Beemster—A. Rozendaal: *Potato viruses: properties and symptoms*) data can be found on viruses transmissible both mechanically and by aphids and other insects as well as on mycoplasmas transmissible by leafhoppers. In this chapter the authors discuss potato virus S with viruses transmissible mechanically, while potato virus M with those transmitted by aphids, although it is a well-known fact that some strains of both viruses can be transmitted by aphids. Perhaps it would have been more correct to discuss potato virus S too with viruses transmissible by aphids. It would also have been better to present the cryptograms of the viruses beside their common English name instead of the binominal nomenclature hardly used today. In the same chapter the authors again deal with the soil-borne viruses, although they were already discussed in Chapter 4. It is remarkable that while potato virus X is mentioned in Chapter 4 as a soil-borne virus, Chapter 10 does not even speak of it. It might be misleading that in Chapter 10 the authors discuss potato (Andean) latent virus in the group of "Potato viruses transmitted by other insects", although it is known that this virus cannot be transmitted by insect vectors. Chapter 11 (A. B. R. Beemster: *Virus translocation in potato plants and mature plant resistance*) presents the differences related with the translocation of the individual viruses within the same host plant on one hand, and the translocation of a single virus in the same host plant, on the other. Chapter 12 (A. J. Reestman: *Incidence of infection in commercial crops and consequent losses*) deals with the yield losses and secondary infections caused by viruses. Chapter 13 (Frederika Quak: *Therapy*) discusses the methods of heat treatment and meristem cultures. Chapter 14 (A. Schepers: *Control of aphid vectors in the Netherlands*) deals with the effects of insecticides, mineral oil, aluminium foil and soil insecticides. Chapter 15 of the book (H. T. Wiersema: *Breeding for resistance*) mainly discusses the problems of hypersensitivity and immunity, but also deals with the inheritance and nature of resistance. Chapter 16 (D. E. van der Zaag: *Dutch techniques of growing seed potatoes*) describes the methods of seed-potato growing in Holland. Chapter 17, the last chapter of the book (J. Hiddema: *Inspection and quality grading of seed-potatoes*) presents methods for the laboratory- and field inspection of seed-potatoes. In the last section of the book there are data on the origin and resistance of potato varieties. The index at the end of the book gives help in looking up concepts and content elements.

The editor and the authors deserve credit for publishing this book, and full homage is rendered to the PUDOC publishing house for the printing work.

J. Horváth

A. Szántó (ed.): *Handbook of agricultural chemization*. Műszaki Könyvkiadó, Budapest, 1972.

The paper collection published by the "Műszaki Könyvkiadó" (Technical Publishing House) gives overall information primarily to agriculturists on the products of the Hungarian chemical industry at the service of agricultural production development. The purpose of the carefully prepared and compiled handbook is to increase the economicalness of agricultural production by a

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tabulated documentation of confirmed data and by the detailed presentation of technical alternatives for supporting the development programmes. The handbook is divided into the following chapters:

N. Mergenthaler—F. Nagymihály: (1) *Methods of supplying nutrients for plants.* The authors describe the nutrient uptake of plants, the influence of soil on nutrient uptake, the forms of nutrients (macro-, meso- and micro-elements), the organic matter supply and fertilization with the aid of a number of well arranged tables. When arranging the data they compare the products of the Hungarian chemical industry and their agricultural utilization with those of the developed capitalist countries. Emphasis is laid in the chapter on the detailed discussion of the importance of solid and liquid fertilizers composed of various active ingredients.

N. Mergenthaler—F. Nagymihály: (2) *Nutrient requirements of plants.* Besides the chemical composition of agricultural and horticultural plants (plant organs) factors influencing the nutrient uptake and the nutrient requirements of cultivated plants are discussed in correlation with the prospective yields.

N. Mergenthaler—F. Nagymihály: (3) *Time and method of fertilization.* The chapter makes directly utilizable proposals on the application of macro-, meso- and micro-elements with the view of improving the cultural practices of up-to-date agricultural units. In addition to basic fertilization and top dressing the tabulated data give detailed information on the modern techniques of liquid- and spray fertilization (microelement application) too.

F. Hargitai: (4) *Chemical plant protection.* The chapter begins with a discussion of the extent of crop failures caused by pathogens, pests and weeds, emphasizing the practical importance of plant protectives in increasing the reliability of production. The wide range of Hungarian made plant protectives (fungicides, insecticides, herbicides) as well as their increased utilization are shown in tables.

P. U. Kralovánszky: (5) *Importance of chemical products in livestock farming.* First the economic factors of producing protein products of animal origin, then the importance of materials produced by chemical industry processes for feeding purposes: yeast, non-protein nitrogen (NPN), amino acids, bioactive additives (vitamins, hormones, enzymes, antibiotics) and of other materials are dealt with. The practical utilization of non-protein nitrogens (NPN) replacing protein and of limiting amino acid supplements, as well as the economical production of proteins of animal origin through biological effectivity are discussed in full detail. The new methods described — modern as to their conception and normative in the up-to-date technology of application — represent an important tendency when the production of animal proteins is to be increased.

A. Szabó: (6) *Plastics in agriculture.* The author discusses the role of Hungarian made plastics in the technical development of agriculture and gives examples of the special application of plastic films, pipes, tubes, containers, bonamide elements, etc. with numeros text illustrations. Various types and technical data of plastic covered forcing houses, methods of lining reservoirs and canal systems, utilization of plastic elements employed in irrigation systems, etc. are described with the special requirements of agriculture taken in consideration.

G. **Mágori**: (7) *Appendix*. Major data of temporarily and permanently licensed plant protectives (fungicides, insecticides, herbicides, adhesives, growth regulators, etc.), chemical designation of active ingredients, main fields of utilization and doses of plant protectives as well as the rates of conversion are presented in clearly arranged tables easy to survey.

The handbook dealing with the agricultural aspects of the Hungarian chemical industry plays an important role in increasing the rentability of agricultural production by giving the agricultural experts information on the range of fertilizers and plastics and on the technologies of application.

B. I. Pozsár

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Official publication of the
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EUPHYTICA

Netherlands Journal of Plant Breeding

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Vol. 23 (1974) (744 pages) contains 94 articles. Some are:

Aspects of cross-pollination in wheat, Cytoplasmic male sterility in ryegrasses, Mutation breeding, Chemical induction of sterility in wheat, Genetical studies of quantitative characters in peas, A study on heterosis in rice, Inheritance of resistance to Pseudoperonospora cubensis in cucumber, The use of aseptic meristem culture in cauliflower breeding, Apomixis and heterogamy in rose rootstocks, The genetic variability of the yield components in pyrethrum, Inheritance of resistance to stripe rust in Triticum, Matromorphy in Brassica oleracea, Breeding research on rose pigments, The occurrence of self-incompatibility in cauliflower, Genome analysis of Eleusine, Assessment of serological techniques for S-allele identification in Brassica, Chromosome behaviour and sterility in Allium hybrids, Potentials of protoplast culture work, Gca and sca in lucerna.

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PRODUCTIA VEGETALA — CEREALE ȘI PLANTE TEHNICE

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The magazine publishes works concerning the problems of the development of the agricultural production (original research, papers drawn up on the basis of experiment and of the scientific literature and achievements of the foremost agricultural units) in the fields of economy and organization of the production, utilization of the stock of land, plant amelioration, agricultural technics, phytotechnics, plant protection, industrialization, mechanization. The original papers are accompanied by Russian, English and French summaries.

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Quartely Journal of Soil Science, Agricultural Chemistry, Fertilization, Soil Biochemistry, Soil Microbiology and Plant Physiology

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